cphst

# Biological Control Unit


# 2009 Annual report

Cover Image Artist:  Joel Floyd (PPQ NIS)

CPHST Biological Control Unit

# 2009 Annual Report

U. S. Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection & Quarantine
Center for Plant Health Science & Technology

CPHST WEBSITE: http://www.aphis.usda.gov/plant_health/cphst/index.shtml

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# SUMMARY OF ACCOMPLISHENTS

In 2009, CPHST employed 16 scientists that focused part or all of their time developing technologies that allow living biological organisms, such as natural enemies and competitors, to effectively mitigate the impacts of introduced, invasive insect pests, weeds, and plant pathogens, while minimizing impacts on the environment and non-target organisms. We received $3,300,000 of Biocontrol Line Item funding, including $50,000 of redirected funds from PPQ-EDP. The majority of these funds (ca. 80%) were used to support CPHST scientists directly working on biological control projects. The remaining 20% was used for equipment, supplies and cooperative agreements.

CPHST scientists provide technical oversight and expertise to programs to ensure that scientific knowledge gaps are identified and addressed, and that developed technologies are transferred to stakeholders as quickly as possible. Our scientists provide support in the discovery and evaluation of new biological control agents offshore and domestically; develop cost effective rearing and monitoring systems for approved biological control agents and their hosts; provide permitted biological control agents collected from established field insectaries and laboratory colonies to PPQ project managers and other project cooperators for redistribution; ensure the safety of biological control agents through on-going post-release monitoring; and develop educational and programmatic materials for use by PPQ and other collaborators.

WEEDS:
CPHST Ft. Collins renewed a cooperative agreement with CABI Europe-Switzerland through FAS for foreign exploration and offshore host range testing of insect biological control agents attacking garlic mustard, yellow toadflax, hoary cress, hound's-tongue, Russian knapweed, dyer's woad, perennial pepperweed, field bindweed and Canada thistle. These weeds were identified as high priority targets by Eastern and Western Regional Program Managers after canvassing State Plant Health Directors and other entities in each state. CPHST is partnering in its support for these weed biological control targets with USDA-ARS, University of Idaho, Colorado State University, and the North American hounds-tongue biocontrol consortium. In anticipation of receiving permitted agents for release from these efforts, CPHST is collecting pre-release data such as percent cover, density and height of plants at chosen sites in the US.

PPQ issued a permit for US field release of a second Russian knapweed biological control agent, the gall midge *Jaapiella ivannikovi*, in early 2009. A colony on this agent was established at the CPHST Mission quarantine facility in TX with help from the CPHST Ft. Collins laboratory and individuals were provided to the CO Department of Agriculture for mass-rearing in Palisade, CO. Initial releases of the gall midge were made at sites in Colorado, Wyoming, and Montana. The release program is a collaborative effort of the CPHST Fort Collins and Mission labs, PPQ Western Region, Colorado Department of Agriculture, University of Wyoming, Montana State University, and the Fremont County (WY) Weed and Pest District.

CPHST Ft. Collins initiated a Colorado-based rearing effort with a yellow toadflax adapted strain of the stem-mining weevil *Mecinus janthinus* in June 2009. This project originated with insects from a newly-discovered weevil population surviving on yellow toadflax (*Linaria vulgaris*) in Montana; normally, this weevil only attacks the related Dalmatian toadflax (*L. dalmatica*). This project is a collaborative effort among the CPHST Fort Collins Lab, Colorado State University (greenhouse-based rearing) and the Colorado Department of Agriculture (field cage rearing).

CPHST Ft. Collins assisted Regional Program Managers and State Departments of Agriculture in the collection and redistribution, as well as evaluation of spread and efficacy, of already approved and existing biological control agents for salt cedars, field bindweed , leafy spurge, and spotted knapweed.

CPHST Ft. Collins continued to assess the distribution and impact of adventive (unintentionally introduced) biological control agents for Canada thistle and hounds-tongue. Both the mite *Aceria anthocoptes* on Canada thistle and the root weevil *Mogulones cruciger* on hound's-tongue were shown to have significant non-target effects. Therefore, recommendations were made not to actively use and redistribute these agents.

CPHST Ft. Collins initiated efforts to survey for and assess the efficacy of plant pathogens attacking Canada thistle and perennial pepperweed in collaboration with CABI Europe-Switzerland and the University of California, respectively. Initial results of an isolate of *Albugo candida*, which causes white rust, collected from perennial pepperweed in CO indicate that the pathogen may be a new race because it is not one of the 11 reported races occurring on a range of weeds and crop species in the United States. Currently, host specificity testing with this isolate and an isolate from California is underway.

CPHST Miami determined that the biological control agent *Gratiana boliviana* of tropical soda apple was present in sites north of Montgomery Alabama two years after release, thus documenting that the beetle can establish in areas that receive periodic snowfalls. They also co-organized and participated in a Florida survey with scientists and government personal from ARS, FL DOACS-DPI and the University of Florida to determine the distribution and natural spread of *G. boliviana* at north Florida release sites. The information gathered from this effort will be used to target future releases of the biological control agent and determine if further mass production is warranted. CPHST Mission also determined that *G. boliviana* could overwinter in East Texas.

INSECTS:
CPHST Phoenix continued the team effort with ARS, Sidney, Montana and Utah State University to discover and develop biological control treatments as alternatives to chemical insecticides for controlling grasshoppers and Mormon crickets on rangeland. In 2009, two major field studies were conducted using previously collected domestic fungi, pathogenic for Mormon crickets. Candidate formulations were applied using the Field Aerial Application Spray Simulation Tower Technique, developed in Phoenix, to 1,104 mini rangeland plots (684 in Sidney, MT and 420 in Logan, UT). The studies compared the activity of three domestic strains of *Metarhizium anisopliae* against Mormon crickets, provided initial dose ranges for future evaluation, and evaluated the persistence of the most promising strain.

CPHST Guatemala continued to conduct field tests to determine whether combined large-scale releases of the egg-parasitoid *Fopius ceratitivorous* and sterile Mediterranean fruit flies will result in a greater than additive suppression of fly populations in the highlands of Guatemala, and, whether this level of suppression is superior to that obtained by the traditional control method of insecticide bait-sprays followed by sterile male releases only. Previous laboratory and field cage studies have suggested that the egg parasitoid can be very effective at controlling medfly populations. This effort is being conducted in conjunction with MOSCAMED program managers from the USA, Guatemala and Mexico.

CPHST Mission partnered with the ER to initiate development of biological control management options for the Harrisia mealybug, which is seriously impacting cactus in Puerto Rico, and *Iceria genistae,* an exotic scale insect in Florida that has the potential to become a serious pest in important agricultural crops such as soybeans, peanuts, and peppers. These efforts are being conducted in collaboration with individuals at the University of Puerto Rico, Puerto Rico Department of Agriculture, Barbados Ministry of Agriculture, EMBRAPA Brazil, APHIS-IS and the State Plant Health Director's offices from Florida and Puerto Rico.

CPHST Mission lead methods development activities for mass-rearing the parasitoid *Tamarixia radiata* as a biological control tool for suppressing the Asian citrus psyllid. Methods for mass production of *T. radiata* on both mature citrus trees and under greenhouse conditions using orange jasmine plants were assessed. In addition, a colony of a newly collected strain of *Tamarixia radiata* from Punjab was established and is being reared and assessed in the CPHST Mission Arthropod Quarantine Facility.

CPHST Miami teamed with the ER to support cooperators at the University of Florida who are evaluating a promising predatory mite for control of the red palm mite and a lady beetle to control the Asian cycad scale, which is killing king and queen sago "palms" and other cycads in Florida and Texas.

CPHST Miami, in collaboration with cooperators at Florida A&M University, ARS and the University of Florida, continued to study natural enemies that are controlling the passionvine mealybug in Trinidad and Puerto Rico. This pest is considered a serious risk to the US similar to that of the pink hibiscus mealybug. These studies have determined that two parasitoids, *Leptomastix dactylopii* and *Coccidoxenoides perminutus*, are largely responsible for keeping the pest below economic injury levels. The parasitoids are available commercially and the information gained through these studies is helping program managers to develop management strategies following the recent detection of this pest in HI. Additionally, experiments were conducted to determine the temperature tolerance of the passionvine mealybug in order to better predict its potential distribution in North America.

CPHST Miami in collaboration with APHIS-IS Caribbean Area, Florida A&M University, Dominican Institute for Agriculture and Forestry Research (IDIAF), and Dominican Republic Ministry of Agriculture (Departamento de Sanidad Vegetal) initiated studies on the coffee mealybug (*Planococcus lilacinus*), an invasive pest of citrus, grape, potatoes, mangos, and guava, in the Dominican Republic; and, in collaboration with APHIS-IS Caribbean Area, Florida A&M University, Aruba Dept. of Agriculture, Husbandry & Fisheries, and Curacao Dept. of Agriculture, Husbandry & Fisheries, began studies on the red palm weevil (*Rhynchophorus ferrugineus*), an economic pest of palms, in the Netherland Antilles, to determine their potential impact and search for local natural enemies.

CPHST Otis has been cooperating with scientists at USDA FS and ARS to develop biological control options for the ash borer (EAB). In 2009, methodologies for rearing *Spathius agrili*, a larval parasitoid of EAB, and EAB itself were transferred to the biological control rearing facility in Brighton MI. Over 6,000 *S. agrili* were released in 2009 in MI, OH, and MD. Emergence traps were developed to allow for non-destructive sampling to determine parasitoid reproduction and establishment. CPHST Otis also discovered new natural enemies of EAB in the Russian Far East, including possibly new species of *Spathius and Tetrastichus*. These parasitoids are currently being reared and evaluated in Otis quarantine facility.

CPHST Otis mass reared Sirex nematodes and carried out experimental field releases of the nematodes against the Sirex wood wasp. *Sirex noctilio,* in New York and Pennsylvania. It was determined that nematode infection rates of the wasp increased following the experimental releases, indicating that the nematodes established and reproduced in North American pine species. Laboratory studies were also initiated to enhance and optimize the mass rearing of Sirex nematodes.

CPHST Albany continued to rear Light Brown Apple Moth (LBAM) life stages in support of biological control efforts by cooperators at CDFA and UC Berkeley. CPHST also cooperated with CDFA on biological control of LBAM utilizing commercially available and native Trichogramma egg parasitoid species.

CPHST Albany continued to make improvements to an artificial diet for mass rearing *Cyphocleonus achetes,* a root feeding weevil used for biological control of knapweeds. To date, 9 consecutive generations of the weevil have been reared on the diet. This diet is already in use in multiple insectaries for mass production of the weevil *H. transversovittatus*. More recently, the diet has been used to rear the red palm weevil, Rhynochophorus ferugineus, as well as a Cerambycid longhorn beetle that is a potential biological control agent for perennial pepperweed. The diet and associated rearing system were published in 2009.

CPHST Gulfport continued to support and manage field releases of phorid flies for biological control of imported fire ants (IFA). Cooperators on the project include APHIS EDP, APHIS ER, ARS, FL-Division of Plant Industries, and many state cooperators. Since 2001, two species of *Pseudacteon* sp. flies have been released at numerous sites in all quarantined states in the contiguous southeastern states and Puerto Rico. In 2009 it was determined that *P. tricuspis* is well established in the southern areas of the IFA regulated area (AL, FL, GA, LA, MS, TX and PR), and moderately established in AR, NC and SC. *P. tricuspis* was not found to be established in CA, OK or TN. The second species, *P. curvatus*, was found to be moderately well to well established in all southern states and PR (AL, AR, FL, FL, LA, MS, NC, OK, SC, TN, TX, and PR). *P. curvatus* has not been released in CA. A publication on the known U.S.-wide distribution of these two species of flies was prepared.

# Biological Control of Canada Thistle

LOCATION: Fort Collins Lab
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Canada thistle, *Cirsium arvense* (Asteraceae), is among the most damaging exotic weeds in the US. Biological control remains a potentially useful management tool that has achieved only sporadic success against Canada thistle infestations. The search for new, effective agents continues. This report summarizes 2009 activities with a native lace bug and an accidentally-introduced rust mite that feed on Canada thistle in the US, and assesses the utility of these arthropods in a biocontrol program.

Assessment of a native lace bug as a potential Canada thistle biocontrol agent. In 2008, I began initial studies with an endemic lace bug feeding on Canada thistle foliage in eastern Colorado, occasionally causing leaf and shoot mortality (CPHST FCL 2008 Annual Report). This insect has been tentatively identified as *Corythucha distincta* (Hemiptera: Tingidae), the distinct lace bug. Host utilization studies with the lace bug and various nontarget plants continued in 2009 with laboratory and field cage tests. In general, my goal was to compare feeding and survival on Canada thistle with that on native and crop plants.

A *C. distincta* laboratory colony was initiated in fall 2008, utilizing adult bugs collected at several Colorado sites. Bugs were placed on Canada thistle plants within Plexiglas cages, and maintained in a growth chamber (day and night temperatures of 25° and 15°C, respectively, and a 16:8 hour light:dark cycle) or in the lab near a window (under ambient temperature and light conditions). I was able to culture three lace bug generations in 2009, though populations varied considerably through the year. In general, only adult lace bugs from the colony were used in the following tests, though field-collected adult bugs were sometimes used to supplement numbers in summer tests. Test plants were grown from seeds in the lab, under ambient temperatures and fluorescent lighting (16:8 hours light:dark).

**Figure 1.** Leaf cages used in the lace bug survival experiment.

*Survival test*. Four native thistles (*Cirsium calcareum*, *C. centaureae*, *C. scariosum*, and *C. scopulorum*) and the exotic weed Canada thistle (*C. arvense*) were used in this study; *C. scariosum* was replicated three times while single plants of the other species were used. Ten adult lace bugs were confined on a large leaf of each test plant, using a cylindrical cage (Fig. 1). Plants were held in a growth chamber under conditions described above, and cages were examined at 7 and 14 days. The numbers of live lace bugs on plant tissue or the cage, dead bugs and missing bugs were recorded. In general, *C. distincta* survival and location patterns on Canada thistle and native thistles were similar, with the exception of comparatively low survival on the native *C. calcareum* after 14 days (Fig. 2).

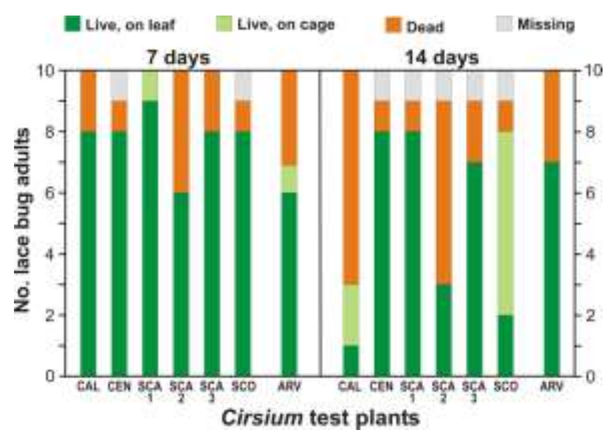


**Figure 2.** Survival and location of lace bug adults at 7 and 14 days (Hosts: CAL = *Cirsium calcareum*; CEN = *C. centaureae*; SCA = *C. scariosum*; SCO = *C. scopulorum*; ARV = *C. arvense*).

*Laboratory host choice tests*. Six test plants were randomly placed in a Plexiglas cage, around a central lace bug release point. This experiment was replicated three times, with various combinations of test plants depending on availability. Test plants included native thistles (*Cirsium calcareum*, *C. centaureae*, *C. ochrocentrum*, *C. tracyi*, and/or *C. undulatum*), the exotic weed milk thistle (*Silybum marianum*), safflower (*Carthamus tinctoria*), cardoon or Spanish artichoke (*Cynara cardunculus*), and garden sunflower (*Helianthus annuus*). Each replicate also used a Canada thistle plant. Lace bug adults were released in the center of each cage; cages were maintained in a growth chamber with a 16:8 hour photoperiod and 25°:15°C thermoperiod.

All test plants were examined for live bugs at various dates after release. The relative level of lace bug feeding activity (none, slight, moderate, or significant) was noted for test plants in replicate 1. Replicates 1, 2, and 3 employed 30, 50, and 30 *C. distincta* adults, respectively.

Utilization of native thistles was similar to, or exceeded, utilization of Canada thistle (Tables 1 & 2). In general, crop test plants (cardoon, safflower, and sunflower) and milk thistle were not, or rarely, visited by *C. distincta*.

**Table 1.** *Corythucha distincta* laboratory host choice test, rep. 1: adult location and feeding (N = native plant).

| **Test plant** (N = native) | **7 d** | | **14 d** | | **24 d** | |
|---|---|---|---|---|---|---|
| | # live bugs on plant | Leaf feeding | # live bugs on plant | Leaf feeding | # live bugs on plant | Leaf feeding |
| *Cirsium calcareum* (N) | 3 | Slight | 2 | Slight | 0 | Slight |
| *C. centaureae* (N) | 9 | Moderate | 11 | Significant | 2 | Significant |
| *C. ochrocentrum* (N) | 2 | Slight | 1 | Slight | 0 | Slight |
| *C. tracyi* (N) | 1 | None | 5 | Slight | 1 | Slight |
| *C. undulatum* (N) | 0 | None | 0 | None | 2 | Slight |
| *Cirsium arvense* | 1 | None | 0 | None | 0 | None |
| **Total** | **16** (of 30) | | **19** (of 30) | | **5** (of 30) | |

**Table 2.** *Corythucha distincta* laboratory host choice test, reps. 2 and 3: adult location (N = native plant).

| **Test plant** (N = native) | **Rep. 2** | | **Rep. 3** | | |
|---|---|---|---|---|---|
| | **7 d** | **24 d** | **3 d** | **9 d** | **16 d** |
| | # live bugs on plant | # live bugs on plant | # live bugs on plant | # live bugs on plant | # live bugs on plant |
| *Cirsium calcareum* (N) | | | 2 | 2 | 0 |
| *Cirsium tracyi* (N) | | | 5 | 5 | 2 |
| *Cirsium undulatum* (N) | 0 | 3 | 0 | 2 | 0 |
| *Carthamus tinctoria* | 0 | 0 | | | |
| *Cynara cardunculus* | 1 | 0 | 0 | 0 | 0 |
| *Helianthus annuus* | 0 | 0 | | | |
| *Silybum marianum* | 0 | 1 | 0 | 0 | 0 |
| ***Cirsium arvense*** | **11** | **1** | **0** | **0** | **0** |
| **Total** | **12** (of 50) | **5** (of 50) | **7** (of 30) | **9** (of 30) | **2** (of 30) |

*Field cage host choice tests*. Nine test plants were planted within two 1-m$^3$ fine-mesh cages at the ARS farm (Fig. 3). These included several native *Cirsium* thistles, Canada thistle, milk thistle, sunflower, cardoon, and safflower.

**Figure 3.** Field cages used in the host-choice test.

One hundred *C. distincta* adults were released in each cage. All plants were examined 7, 14, and 21 days later and all bugs counted (Table 3, following page). Counts were low in both cages; ants were abundant on test plants, many of which were infested with aphids, so predation was probably an issue. Once again, lace bug counts on native thistles were similar to, or higher, than on Canada thistle. Bugs were not observed on safflower or sunflower plants, and a single bug was observed twice on cardoon.

*Corythucha distincta* is apparently a native North American insect. Previous reports had listed Canada thistle and other exotic *Cirsium* and *Carduus* thistles (not tested here), two native *Cirsium* thistles, as well as plants from Fabaceae, Malvaceae, and several other plant families as hosts. In general, many lace bugs are believed to have

**Table 3.** *Corythucha distincta* field cage host choice test: adult bug location.

| **Test plant** (N = native) | **Cage 1** | | | **Cage 2** | | |
|---|---|---|---|---|---|---|
| | **7 d** | **14 d** | **21 d** | **7 d** | **14 d** | **21 d** |
| | # live bugs on plant | # live bugs on plant | # live bugs on plant | # live bugs on plant | # live bugs on plant | # live bugs on plant |
| *Cirsium calcareum* (N) | 2 | 0 | 0 | 9 | 4 | 0 |
| *Cirsium ochrocentrum* (N) | 2 | 1 | 4 | 5 | 9 | 4 |
| *Cirsium tracyi* (N) | 6 | 13 | 7 | 8 | 8 | 3 |
| *Carthamus tinctoria* | 0 | 0 | 0 | | | |
| *Cynara cardunculus* | 1 | 0 | 0 | 0 | 1 | 0 |
| *Helianthus annuus* 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Helianthus annuus* 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Silybum marianum* 1 | 2 | 0 | 0 | 5 | 4 | 3 |
| *Silybum marianum* 2 | | | | 0 | 1 | 0 |
| ***Cirsium arvense*** | **5** | **3** | **7** | **0** | **1** | **1** |
| **Total** | **18** (of 100) | **17** (of 100) | **18** (0f 100) | **27** (of 100) | **28** (of 100) | **11** (of 100) |

comparatively narrow host ranges. From these experiments, it appears that native *Cirsium* thistles could be the ancestral hosts, with closely-related exotic thistles also utilized. More distantly-related plants in Asteraceae (e.g. sunflower, safflower, and cardoon) appear to be poor hosts, at best; host records from other plant families are probably erroneous. Though *C. distincta* may occasionally cause significant feeding damage to Canada thistle populations, its utilization of native *Cirsium* thistles should eliminate its use as a biocontrol agent.

Utilization of native *Cirsium* thistles by the Canada thistle rust mite. Efforts to document nontarget utilization of native thistles and related plants by *Aceria anthocoptes* continued in 2009. Sixteen thistles were sampled for mites in 2009, including ten native *Cirsium* thistles and four exotic species. Among the native thistles, three species were sampled for the first time: *Cirsium canescens* (collected in Laramie Co., Wyoming), *C. eatonii* (tentative identification; Summit Co., Colorado), and *C. neomexicanum* (Mesa Co., Colorado). Putative *A. anthocoptes* mites were collected from all native thistles except *C. neomexicanum* (Table 4). 2009 sampling confirmed that no mites have been collected from the exotic weed bull thistle (*Cirsium vulgare*) or from any thistles outside the genus *Cirsium*.

| | ***Aceria* mites coll'd (year)** | | | | |
|---|---|---|---|---|---|
| **Host plant** | **2006** | **2007** | **2008** | **2009** | **Positive ID?** |
| **Native thistles** | | | | | |
| *Cirsium barnebyi* | | | + | + | **Yes** |
| *C. calcareum* | | + | + | + | **Yes** |
| *C. canescens* | | | | + | **?** |
| *C. centaureae* | + | + | + | + | **Yes** |
| *C. eatonii* (?) | | | | + | **?** |
| *C. neomexicanum* | | | | – | |
| *C. ochrocentrum* | | + | + | + | **Yes** |
| *C. scariosum* | + | + | + | + | **Yes** |
| *C. scopulorum* | + | + | + | + | **Yes** |
| *C. tracyi* | | – | + | + | **No (?)** |
| *C. undulatum* | + | + | + | + | **Yes** |
| **Exotic thistles** | | | | | |
| *Cirsium arvense* | + | + | + | + | **Yes** |
| *C. vulgare* | – | | – | -– | |
| *Carduus nutans* | – | – | | – | |
| *Onopordum acanthium* | | | – | – | |

**Table 4.** Collection and identification of *Aceria anthocoptes* from various hosts in Colorado and Wyoming.

Additional voucher specimens were identified by Dr. Radmila Petanovic, University of Belgrade (Serbia) in 2009. These confirm that mites from seven of the native *Cirsium* thistles and from Canada thistle are *A. anthocoptes*; the identification of mites collected from *C. tracyi* remains uncertain (Table 4). 2009 mite samples from *C. canescens* and *C. eatonii* will be sent to Serbia for identification in 2010.

Our thistle garden, initiated in 2008, was sampled for *A. anthocoptes* abundance during summer and fall 2009, to ascertain among-host differences in relative mite abundance and seasonal population patterns under field conditions. The garden contains seven native *Cirsium* thistles, the exotic weeds Canada thistle, bull thistle, and milk thistle, and the crop plants globe artichoke (*Cynara scolymus*) and cardoon. Because there were insufficient plants of most species for replication, five leaves were removed from a single plant of each species at weekly or biweekly intervals. Fresh leaf samples were weighed, and mites were extracted using a bleach solution (CPHST FCL 2008 Annual Report) and counted; mite numbers were divided by sample biomass to account for among-host differences in leaf size. In general, mites were most abundant on Canada thistle and native thistles, with the highest numbers recorded from *Cirsium scopulorum* (Fig. 4). Populations appeared to 'peak' in late August-early September. A few mites were occasionally collected from milk thistle, artichoke, and cardoon, but virtually no mites were found on bull thistle throughout the season.

Results from 2009 confirm earlier observations that; (a) *Aceria anthocoptes* only utilizes thistles in the genus *Cirsium*; (b) at least 10, and undoubtedly more, native *Cirsium* thistles are hosts, and (c) the European thistle *Cirsium vulgare* is not a mite host, corroborating European observations. Thus, the utilization of nontarget native plants precludes the use of this adventive mite as a Canada thistle biological control agent.

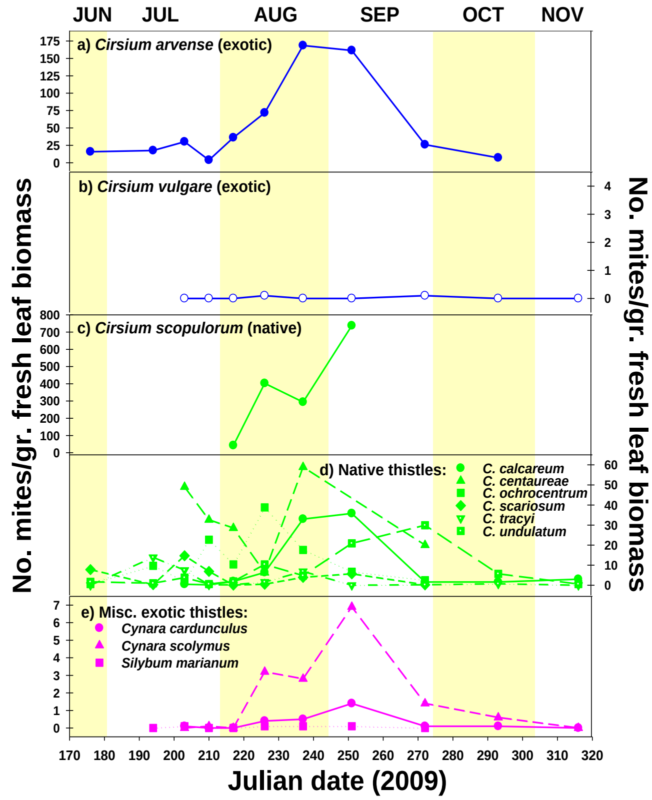


**Figure 4.** Relative abundance of *Aceria anthocoptes* on 12 potential hosts in ARS farm thistle garden (2009).

## Updates on Selected PPQ Weed Biocontrol Projects

LOCATION: Fort Collins Lab
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### Biological control of saltcedars (*Tamarix* spp.)

*Distribution of saltcedar leaf beetles*. In 2009, there were no new *Diorhabda* leaf beetle releases or augmentation of existing field insectaries in the 10 states involved in PPQ's northern US release program. This was a consequence of an ongoing legal action addressing possible non-target impacts of *Diorhabda* spp. in the southwestern US, and reinitiation of program consultation between USDA-APHIS and USDA Fish and Wildlife Service (FWS). It is uncertain if, or when, active beetle distribution will be resumed. However, post-release monitoring efforts continued at most sites in 2009.

*Phenology of saltcedar leaf beetle life stages*. In 2008, we initiated a study to document the relative abundance of *Diorhabda* leaf beetle life stages throughout the growing season. These data were collected at two locations: a field insectary site near Torrington, Wyoming and caged populations released near Fort Collins, Colorado (CPHST Fort Collins 2008 Annual Report). Beetle data would be coupled with temperature data collected at both locations in an attempt to develop degree day-based *Diorhabda* phenology models, which could be used by cooperators to schedule beetle sampling and collection efforts.

However, no beetles from any life stage were collected at the Wyoming site in spring and summer 2009; we believe that this population no longer survives. Similarly, no beetles of any life stage were collected in the three Fort Collins cages from spring through fall 2009. *Diorhabda* adults were abundant in all three cages in late summer 2008, and presumably some or all of these insects entered diapause. Since no adult beetles emerged in the spring, it appears that the caged *Diorhabda* populations did not survive the winter. Perhaps the overwintering substrate available in the cages was inadequate to protect beetles from low winter temperatures and moisture levels. We are attempting to locate other *Diorhabda* populations to possibly resume this study in 2010.

### Biological control of Russian knapweed (*Acroptilon repens*)

*Initial field releases of the bud gall midge Jaapiella ivannikovi*. *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) was permitted for US field release in early 2009. Releases were made on the Wind River Reservation near Riverton, Fremont County, Wyoming in May and July 2009. These releases utilized mature *J. ivannikovi* galls, containing pupae, from a quarantine colony established at Montana State University (MSU) (Fig. 1). One caged and two open releases were made in May and five additional open releases were made in July, for a total of eight separate releases within about a 3-ha area. *J. ivannikovi* has several generations per year; monitoring visits throughout the summer discovered progeny galls within the field cage and in the vicinity of the various open releases (Fig. 2). In August 2009, additional galls from MSU were released at a site near Powell, Park Co., WY (also an open release); site visits in September confirmed post-release galling on Russian knapweed plants. Thus, one caged and seven open *J. ivannikovi* releases were made in two Wyoming counties in 2009. Post-release sampling showed establishment at both sites; 2010 monitoring will confirm winter survival. If populations survive and spread in 2010, gall collections may be made for distribution in other areas of Wyoming and in other western states. Partners in the Wyoming releases include the PPQ Wyoming state office, Fremont Co. Weed and Pest, the U. of Wyoming, and Montana State U.

**Figure 1.** *Jaapiella ivannikovi* galls released in Fremont Co., WY, May 2009.

**Figure 2.** Russian knapweed galled by *Jaapiella ivannikovi*, Fremont Co., WY, June 2009.

*Jaapiella ivannikovi* was also released in a field cage at the Colorado Department of Agriculture (CDA) laboratory in

Palisade, Mesa Co., Colorado in August 2009. Galls used in this release originated from a quarantine colony at the CPHST Mission laboratory. Adults emerged from these galls, but no obvious post-release galling was observed in the cage. CDA will monitor the cage in spring 2010 to determine if *J. ivannikovi* has successfully established.

Galls from the Mission quarantine were sent to CPHST Fort Collins in October 2009, where they were placed in a growth chamber (5.0°:2.5°C day:night temperatures, 12:12 hours light:dark). In early 2010, these galls will be removed from cold storage; any emerging *J. ivannikovi* adults will be used to initiate a laboratory rearing program at FCL.

*Status of the bud gall wasp Aulacidea acroptilonica. Aulacidea acroptilonica* (Hymenoptera: Cynipidae) was permitted for US release in 2008, the first insect approved as a Russian knapweed biocontrol agent. However, difficulties in rearing this insect in quarantine culture (CPHST Mission and MSU) have prevented any US field releases. Pre-release quarantine rearing is required because *A. acroptilonica* galls collected in its native Asian range are typically infested with a variety of parasitoids, predators, or inquilines, preventing the direct release of foreign-collected material. In 2008, a field cage release of the wasp was made by Agriculture and Agri-Foods Canada in Lethbridge, Alberta, but it appears that this population did not establish. We will revisit rearing efforts in 2010, hopefully providing insects for field release in summer 2010.

## Biological control of yellow toadflax (*Linaria vulgaris*)

**Rearing and release of a yellow toadflax-adapted 'strain' of** *Mecinus janthinus*. In 2008 and 2009, established populations of the stem-mining weevil *Mecinus janthinus* (Coleoptera: Curculionidae) were confirmed in yellow toadflax infestations at several western Montana sites. This was unexpected since the preferred host of this weevil is the related Dalmatian toadflax (*L. dalmatica*), and all other *M. janthinus* releases targeting yellow toadflax had failed to establish. This weevil has successfully controlled Dalmatian toadflax at many sites; utilization of the yellow toadflax 'strain' may lead to similar results against this weed in eastern and western states. In June and July 2009, *M. janthinus* adults from yellow toadflax sites were collected in Montana and shipped to CPHST Fort Collins to be used for rearing and research by project partners. Cooperators include the U. of Idaho, Colorado State U. (CSU), Colorado Department of Agriculture, and USDA Forest Service.

In early July 2009, CDA cooperators released approximately 150 *M. janthinus* adults in a field cage at a yellow toadflax site near Meeker, Rio Blanco County, Colorado (Fig. 3). Post-release monitoring revealed the presence of stem mines and progeny larvae, pupae, and adults. If beetles successfully survive the winter, adults will be collected in 2010 to initiate new caged releases in Colorado and, perhaps, other western states.

**Figure 3.** Caged *Mecinus janthinus* release on yellow toadflax, Rio Blanco Co., CO, July 2009.

In June 2009, additional weevils were provided to CSU cooperators for basic research on *Mecinus janthinus* host utilization. Preliminary results from host performance experiments with the yellow toadflax 'strain' of *M. janthinus* indicate that these weevils perform somewhat better on yellow toadflax than on Dalmatian toadflax, but that they can also lay eggs, feed and develop successfully on either host. Thus, there appears to be no inherent reason why the Montana *M. janthinus* population has uniquely established on yellow toadflax; perhaps plant characteristics or physical site factors are involved. CSU will continue these experiments in 2010 in an effort to better understand host selection by *M. janthinus*.

CSU also initiated a *Mecinus janthinus* rearing program in June 2009. Approximately 180 adult weevils were divided among three rearing cages, each containing two potted yellow toadflax plants. At weekly intervals, the exposed plants were removed from each cage and two new yellow toadflax plants were introduced; this continued until the end of July, when all adult weevils had died (*M. janthinus* adults are relatively long-lived, and females lay eggs over an extended period of time). All exposed plants were maintained in an outdoor rearing cage until late November, when the aboveground portions of each plant (containing progeny adults) were harvested and moved into cold storage (1°C). Up to 600 *M. janthinus* adults were produced in 2009; these will be removed from cold storage in late spring 2010 to repeat the rearing effort.

## Biological control of musk thistle (*Carduus nutans*) and Scotch thistle (*Onopordum acanthium*)

I completed a 'white paper' titled *Current status of, and future prospects for, biological control of musk thistle and Scotch thistle in the US*. This report was requested by PPQ Western Region and was delivered to the Regional Program Manager and Regional Biocontrol Steering Committee in November 2009. It reviews the current options for thistle biocontrol and suggested strategies for a renewed biocontrol effort against these damaging exotic weeds.

# Pre-release Research and Development Efforts for PPQ Weed Biocontrol Targets: Project Updates

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Introduction. Before classical biological control can be implemented against an exotic weed target, potential biological control agents must be identified and collected, studied for possible effectiveness, and screened for host specificity (i.e. their risk to nontarget native US and crop plants assessed). Pre-release research and development is conducted in the native range of the weed, typically in Europe and Asia. Host specificity, the relative tendency of a potential agent to restrict its feeding and development to the target weed while avoiding utilization of other plants, is assessed using (a) no-choice (or starvation) tests, where the agent is exposed only to a test plant, and (b) choice tests, where the agent is exposed to one or more test plants in the presence of the target weed.

This report summarizes the current status of research and development efforts addressing 10 weed targets prioritized by PPQ. All were identified during PPQ target canvassing efforts in 1997 (Russian knapweed and Canada thistle), 2000 (field bindweed, hoary cress, and garlic mustard) and 2005 (houndstongue, dyer's woad, perennial pepperweed, yellow toadflax, and hawkweeds). This work has been conducted by CABI Bioscience (Delémont, Switzerland), along with a variety of European and Asian cooperators. USDA-ARS is collaborating in pre-release research addressing hoary cress, Russian knapweed, and hawkweeds. Through a cooperative agreement with CABI, CPHST Fort Collins funded pre-release research for all biocontrol projects listed below (except hawkweed) in 2009. A cooperative agreement with the University of Idaho was funded in 2009 to provide native US plants for host specificity tests with hawkweed agents.

**Dyer's woad,** *Isatis tinctoria* (Brassicaceae). Biocontrol research and development efforts targeting this weed began in 2005, with natural enemies surveyed in western Europe, Turkey, Russia, and Kazakhstan. Due to budget constraints, CABI concentrated developmental efforts with three prospective biocontrol agents in 2009. The root crown-mining weevil *Ceutorhynchus rusticus* is a promising agent with an apparently high degree of host specificity; in tests through 2009, it has been able to complete at least limited development on only 5 of 63 nontarget host plants tested. The stem-mining flea beetle *Psylliodes isatidis* is another potentially effective agent. Host specificity tests to date indicate that 28 of 60 nontarget plants could support some *P. isatidis* larval development in no-choice tests, though utilization is much lower in choice tests. Preliminary host specificity tests with the seed-feeding weevil *Ceutorhynchus peyerimhoffi* began in 2009. Host specificity experiments with all three agents will continue in 2010.

Garlic mustard, *Alliaria petiolata* (Brassicaceae). The biocontrol development effort targeting garlic mustard was initiated in 1998. Host specificity tests with the most promising potential agent, the root-mining weevil *Ceutorhynchus scrobicollis* (Fig. 1), were completed in 2008, and showed that the weevil developed on only 5 of 79 plants studied in no-choice or host choice environments. No native US plants tested were utilized; however, some development occurred on the crop plant watercress, *Nasturtium officinale*. A release petition was submitted to the weed biocontrol Technical Advisory Group (TAG) in 2008, but TAG recommended in early 2009 that *C. scrobicollis* not be released in the US, primarily due to concerns about possible nontarget utilization of several native mustards. CABI plans to conduct additional host specificity experiments and re-submit the petition in 2010.

**Figure 1.** Garlic mustard root weevil, *Ceutorhynchus scrobicollis*, adult (CABI Bioscience).

Other potential garlic mustard agents in development include three additional *Ceutorhynchus* weevils, and host specificity experiments continued in 2009. The shoot-mining weevil *C. alliariae* was able to complete development on 4 of 77 plants tested, including watercress and a common native mustard, *Rorippa sinuata* (spreading yellowcress). Another shoot-mining weevil, *C. roberti*, has developed on only 3 of 64 nontarget plants tested, none of which is a native species. A seed-feeding weevil, *C. constrictus*, only utilized 1 of 72 plants tested to date – the

Eurasian black mustard, *Brassica nigra*, which is a very minor crop plant but also an exotic weed in the US. Host specificity experiments with these three insects will also be continued in 2010.

Hoary cress, *Lepidium draba* (Brassicaceae). Through 2009, seven potential biocontrol agents have been identified and studied by CABI, and four are currently in active development. The agent closest to completion is the stem-galling weevil *Ceutorhynchus cardariae*. This insect was able to complete development on 12 (including 7 US natives) of 93 nontarget plants tested in no-choice experiments but did not develop on any nontarget species in 2009 choice tests. Host specificity tests will be completed, and a release petition submitted to TAG, in 2010. Testing is nearly complete with the seed-feeding weevil *C. turbatus*, which appears to be the most host-specific prospective hoary cress agent. This insect was able to complete development on one nontarget plant tested, the European species *Lepidium campestre* (an exotic weed in the US). Host-specificity tests will be continued in 2010, with submission of a TAG petition in 2010 or 2011. *C. turbatus* should be able to reduce hoary cress seed production and spread but will not kill plants.

The two remaining potential hoary cress agents are the root-mining weevil *Melanobaris* sp. near *semistriata* and the shoot-mining flea beetle *Psylliodes wrasei*. *Melanobaris* sp., the only root feeder in development, has demonstrated larval feeding on 12 of 35 test plants to date, including 5 native species. CABI plans to continue host specificity experiments in 2010, after which a decision on whether or not to continue research with *Melanobaris* sp. will be made. *P. wrasei* appears to be a high impact agent, but with a fairly broad host range. In no-choice larval feeding tests through 2009, 26 of 78 tested nontarget plants supported development, including one native US plant but no crop species. In 2010, a decision will be made on whether to continue development efforts with *P. wrasei*.

In 2010, additional natural enemy surveys for new potential hoary cress agent may be conducted in Iran, Russian, Turkey, and/or Uzbekistan.

Perennial pepperweed, *Lepidium latifolium* (Brassicaceae). In 2006 and 2007, CABI conducted natural enemy surveys in Turkey, Russia, Kazakhstan, and Russia to identify potential perennial pepperweed agents. Preliminary host specificity tests with several prospective agents were conducted in 2008 and 2009. These include *Ceutorhynchus marginellus*, a stem-galling weevil and *Phyllotreta reitteri*, a stem-mining flea beetle (Fig. 2).

*C. marginellus* development occurred on 5 of 32 nontarget plants in no-choice tests, but these plants were attacked much less frequently than the target weed. *P. reitteri* completed development on 15 of 39 plants tested to date, including limited development on 8 native species. Preliminary studies with a third agent, the root-mining weevil *Melanobaris* sp. near *semistriata*, show that this weevil is distinct from the *Melanobaris* weevil feeding on hoary cress. Host specificity testing will continue with these three insects in 2010. Host specificity experiments will begin for two additional Turkish arthropods: the gall mite *Metaculus lepidifolii* and the stem-mining fly *Lasiosina deviata*.

**Figure 2.** Perennial pepperweed flea beetle, *Phyllotreta reitteri*, adult (CABI Bioscience).

Field bindweed, *Convolvulus arvensis* (Convolvulaceae). Two biocontrol agents, the gall mite *Aceria malherbae* and the leaf-feeding moth *Tyta luctuosa* were released in the US in the 1980s, but have had limited impact on most field bindweed infestations. In 2009, CABI and its eastern European partners initiated a new developmental effort, primarily seeking potential root- and stem-feeding bindweed agents. The host plant test list will be revised and submitted for TAG consideration in 2010. Two of the most promising potential new agents are *Melanagromyza albocilia*, a shoot- and root-mining fly, and *Longitarsus pellucidus*, a root-mining flea beetle. Host specificity experiments with these two insects will be initiated in 2010.

Yellow toadflax, *Linaria vulgaris* (Scrophulariaceae). CABI continued research with two promising potential biocontrol agents in 2009: the stem-galling weevil *Rhinusa pilosa* and the stem-mining weevil *Mecinus heydeni*. Host specificity experiments with *R. pilosa* have employed 67 nontarget plants to date, including 41 native North American species. Limited adult development was observed on one native, *Sairocarpus virga*, in no-choice tests but no development occurred on natives in host-choice tests. *M. heydeni* has been tested with 26 plants, including 21 native species; limited development has been observed on two native plants in no-choice experiments. Both agents appear to have a major negative impact on yellow toadflax and are quite host-specific. Hopefully, host specificity experiments can be completed in 2010, if sufficient native test plants are available. Because of recent taxonomic revisions of the

Scrophulariaceae and related families, a revised host plant test list will be completed by 2010.

Houndstongue, *Cynoglossum officinale* (Boraginaceae). In 2009, pre-release research continued with the most promising biocontrol agent, the seed-feeding weevil *Mogulones borraginis* (Fig. 3). This weevil appears to be the most host-specific houndstongue agent, and is able to reduce seed production by up to 50%. An obstacle to possible release in the US has been host-specificity testing with two critical native plants, *Cynoglossum grande* (Pacific houndstongue) and *C. occidentale* (western houndstongue). Flowering and seed production have proven very difficult with these plants in greenhouse and quarantine conditions. In 2009, CABI successfully accomplished feeding tests with the seed weevil and *C. grande*; no oviposition or feeding were observed on this native plant in host choice experiments. These tests will be completed in 2010, leading to a possible submission of a TAG petition later in 2010. Weevils were collected in Europe and sent to quarantine facilities at the University of Idaho and Agriculture and Agri-Foods Canada (Lethbridge, Alberta) in 2009, to initiate colonies for research and field release.

**Figure 3.** Houndstongue seed weevil, *Mogulones borraginis*, adult (CABI Bioscience).

Russian knapweed, *Acroptilon repens* (Asteraceae). Two agents were permitted for US field release in 2008 (the gall wasp *Aulacidea acroptilonica*) and 2009 (the gall midge *Jaapiella ivannikovi*). Work on additional agents continued in 2009. These include the root-boring moth *Cochylimorpha nomadana*; this is a potentially effective agent but is very difficult to work with in the lab or in the field, and CABI is considering suspending research on this insect. The bud gall mite *Aceria* sp. near *acroptiloni* appears to be a host-specific agent, and taxonomic and host specificity studies will continue in 2010. An unidentified leaf-feeding beetle from Uzbekistan will be identified and its biology and host specificity will be examined initially in 2010. Several seed-head gall flies (*Urophora* spp.) have been identified by USDA-ARS; in 2010, life history and host specificity studies will continue in quarantine at Montana State U.

Hawkweeds, *Pilosella* (*Hieracium*) spp. (Asteraceae). CPHST has not directly funded development work at CABI with prospective biocontrol agents, but has funded collection and propagation of native North American test plants used in this research from 2007 through 2009. Orange hawkweed (*Pilosella aurantiaca*) and meadow hawkweed (*P. caespitosa*) are the two primary exotic weeds in the US that are targets for new biocontrol agents. A release petition for the bud gall wasp *Aulacidea subterminalis* was submitted to, and approved by, TAG in 2009; an environmental assessment is currently being prepared by APHIS, and field release may be permitted in 2010. Host specificity experiments with the root-mining fly *Cheilosia urbana* continued in 2009. To date, 63 nontarget plants, including 36 native North American species, have been tested; several native *Hieracium* hawkweeds supported limited development. A release petition may be submitted to TAG in 2010. Host specificity testing will continue with two other prospective agents in 2010: the stem-mining fly *Cheilosia psilophthalma* and the stem gall wasp *Aulacidea hieracii*.

Canada thistle, *Cirsium arvense* (Asteraceae). To date, seven insects and a rust fungus have been deliberately or accidentally introduced and employed as Canada thistle biocontrol agents in the US. However, no current agent is reliably effective, and several have negatively impacted nontarget native *Cirsium* thistles. Recent surveys show that there are few thistle-feeding insects in Europe and Asia sufficiently host specific to be considered as potential biocontrol agents for the US. Thistle pathogens, which may be highly host-specific, have received limited attention for Canada thistle biocontrol. CABI and its partners proposed pathogen surveys in the Xinjiang Autonomous Region of northwestern China in summer 2009; this region supports a diverse *Cirsium* flora and is climatically similar to much of the continental US. However, political unrest in Xinjiang precluded access, so 22 sites in western Mongolia were instead surveyed in 2009. A variety of pathogens were collected, but it appears that the Mongolian hosts were the closely-related *Cirsium setosum* rather than *C. arvense*. In 2010, molecular analyses will be used to confirm populations of *C. arvense* and *C. setosum*; additional pathogen surveys will also be conducted in Xinjiang and, perhaps, Tibet.

## Survey for White Rust (*Albugo candida*) and Other Natural Enemies of Perennial Pepperweed (*Lepidium latifolium*)

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**Figure 1.** White rust pustules on perennial pepperweed.

Perennial pepperweed, an introduced plant in the mustard family (Brassicaceae) from southeastern Europe and Asia, is invasive throughout the western United States. It can establish in a wide range of environments and is a common problem in flood plains, irrigation structures, pasture, wetlands, riparian areas, roadsides, and residential sites. Perennial pepperweed can rapidly form large, dense stands that displace desirable vegetation. Root segments as small as 1 inch are capable of producing new shoots. Perennial pepperweed is a prolific plant; in addition to the root reserves it is capable of producing 6 billion seeds per acre.

Current research focuses on herbicides for pepperweed control. Several postemergent herbicides control perennial pepperweed, but repeat applications are usually necessary for several years to treat resprouting shoots and seedlings. Although there is an active program to find biological controls for perennial pepperweed, there are currently none available. Perennial pepperweed is in the same family as mustard and canola and there is concern that a biocontrol insect or pathogen would attack an agricultural crop.

A white rust disease, characterized by white pustules (Fig. 1) containing sporangia on the underside of the leaves, has been identified on perennial pepperweed plants across the United States, especially during wet years. Some reports state that this *Albugo* reduces seed set and number while others report that this white rust provides little or no control.

The primary goal of this project was to evaluate the efficacy of white rust on perennial pepperweed and to survey for other endemic pathogens of the weed in Colorado and Wyoming at a number of field sites. Determining which natural enemies are already present in the United States and assessing their current and potential impact on the target weed is a logical first step in developing biological control as a viable management option for perennial pepperweed. Of the eleven races of *Albugo candida* reported to cause white rust on a range of hosts within the Brassicaceae in North America, there have not been reports on which race causes white rust of pepperweed. A second goal of this project involved a race determination of the *Albugo candida* on pepperweed and comparison with the races that cause disease on mustard and canola using a host differential.

In 2006 through 2008, 18 field sites in Colorado and Wyoming in five counties were surveyed for the presence of diseased perennial pepperweed. A total of 202 plant samples displaying disease symptoms were collected, plant pathogens cultured and identified. A total of three fungal genera were identified including: *Albugo, Peronospora*, and *Phyllostitca.*

The differential hosts for the 11 known races of *A. candida* were grown and inoculated with the Colorado isolate in 2008 and 2009 (Table 1, following page). We obtained an isolate of *Albugo candida* from perennial pepperweed from California in 2008 and have completed the same inoculations of the species listed in Table 1. Results indicate that the race of *A. candida* affecting perennial pepperweed in Colorado and California is a new or unreported race, because it did not cause disease on any of the reported differentials for races 1 through 11.

Since the Colorado and California isolates did not conform to an existing race of *A. candida*, several native and invasive *Lepidium* species, crop species, and other species within the Brassicacae were grown to begin the preliminary stages of host specificity testing for the white rust pathogen (Table 2, following page). Both isolates have a similar narrow host range that differs slightly. The Colorado isolate infected four hosts in addition to perennial pepperweed including *Iberis umbellata*, *Lepidum ruderale*, *Lepidum sativum*, and *Stanleya pinnata* (shown in red). The California isolate infected these same four hosts and *Lepidium campestre*, *Lepidium draba*, and *Thelypodium integrifolium* (shown in blue).

**Table 1.** Reaction of Colorado and California on host differentials for the 11 reported races of *A. candida.*

| Plant | Cultivar | Tests for Which Race? | Colorado Isolate Reaction R=Resistant S=Susceptible | California Isolate Reaction R=Resistant S=Susceptible |
|---|---|---|---|---|
| *Raphanus sativus* (radish) | French Breakfast | 1 | R | R |
| *Brassica juncea* (brown and oriental condiment mustard) | Mustard, India tendergreen | 2 | R | R |
| | 'Burgonde' | 2A | R | R |
| | 'Cutlass' | 2V | R | R |
| *Armoracia rusticana* (horse radish) | | 3 | R | R |
| *Capsella bursa-pastoris* (shepherd's purse) | | 4 | R | R |
| *Sisymbrium officinale* (hedge mustard) | | 5 | R | R |
| *Rorippa islandica* (water cress) | | 6 | R | R |
| *B. rapa* (*B. campestris*) | | 7 | R | R |
| | Broccoli Raab | 7 | R | R |
| | 'Torch' | 7A | R | R |
| | 'Reward' | 7V | R | R |
| | 'Tobin' | 7V | R | R |
| | 'AC Parkland' | 7V | R | R |
| *B. nigra* (black mustard) | | 8 | R | R |
| *B. oleracea* | | 9 | R | R |
| | Cultivar group acephala (Kale) | | R | R |
| | Cultivar group acephala (Collards) | | R | R |
| | Cultivar group botrytis (Cauliflower) | | R | R |
| | Cultivar group capitata (Cabbage) | | R | R |
| | Cultivar group gemmifera (Brussels Sprouts) | | R | R |
| | Cultivar group gongylodes (Kohl rabi) | | R | R |
| | Cultivar group italic (Broccoli) | | R | R |
| *Sinapis alba* (white mustard) | | 10 | R | R |
| *B. carinata* | | 11 | R | R |

**Table 2.** Reaction of Colorado and California isolates of *A. candida* on native, agronomic, and invasive Brassicaceae. Those species in red are susceptible to both *A. candida* populations, those in blue are susceptible to only one.

| Plant | Colorado Isolate Reaction R=Resistant S=Susceptible | California Isolate Reaction R=Resistant S=Susceptible | Plant | Colorado Isolate Reaction R=Resistant S=Susceptible | California Isolate Reaction R=Resistant S=Susceptible |
|---|---|---|---|---|---|
| *Alyssum saxatile* | R | R | *Lepidium arvense* | R | R |
| *Arabidopsis thaliana* | R | R | ***Lepidium campestre* (field pennycress)** | **R** | **S** |
| *Arabis holboellii var. retrofracta* | R | R | *Lepidium densiflorum* | R | R |
| *Arabis sparsiflora var. subvillosa* | R | R | ***Lepidium draba* (hoary cress)** | **R** | **S** |
| *Barbarea vulgaris* | R | R | *Lepidum fremontii* | R | R |
| *Brassica napus (cvs. Cascade and Bridger)* | R | R | *Lepdium lasiocarpum* | R | R |
| *Brassica rapa subs. pekinensis* | R | R | ***Lepidium latifolium* (Perennial Pepperweed)** | **S** | **S** |
| *Brassica rapa var. rapa* (turnip) | R | R | ***Lepidium ruderale*** | **S** | **S** |
| *Camelina sativa* | R | R | ***Lepidium sativum*** | **S** | **S** |
| *Descurainia californica* | R | R | *Lepidium virginicum var. Pubescens* | R | R |
| *Descurainia incisa subsp. incisa* | R | R | *Lobularia maritima* | R | R |
| *Descurainia californica* | R | R | *Lunaria annua* (money plant) | R | R |
| *Draba reptans* | R | R | *Nasturtium officinale* | R | R |
| *Eruca sativa* (arugala) | R | R | *Sisymbrium altissimum* | R | R |
| *Erysimum capitatum* | R | R | *Stanleya elata* | R | R |
| *Erysimum cheiri* | R | R | ***Stanleya pinnata*** | **S** | **S** |
| *Hesperis matronalis* | R | R | ***Thelypodium integrifolium*** | **R** | **S** |
| ***Iberis umbellata*** | **S** | **S** | *Thelypodium laciniatum* | R | R |

## Biological Control of Tropical Soda Apple in Florida, Georgia, and Alabama using the Leaf-feeding Beetle, *Gratiana boliviana*

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Tropical soda apple (TSA), *Solanum viarum*, is an aggressive perennial plant native to South America. This federally listed noxious weed has caused substantial economic and environmental damage in Southern U.S. with annual control costs estimated at $6.5-16 million. The South American leaf -feeding beetle, *Gratiana boliviana*, was introduced into Florida in 2003 and in Alabama and Georgia in 2004 for biological control of TSA. Through a collaborative effort, the Florida Division of Plant Industry, University of Florida, USDA-ARS, PPQ FL, PPQ GA, Georgia Dept. of Agriculture, and PPQ CPHST Miami developed methods to mass rear, release and evaluate the biological control agent. Since being approved, the group released over 200,000 beetles at more than 350 locations. This technology was subsequently transferred to extension agents, cattle ranchers, conservation organizations, and others as part of an integrated management approach to controlling TSA. The beetle has resulted in substantial suppression of TSA density and fruit production in pastures and natural areas in central and south Florida. In 2009 a multi-agency statewide survey was conducted to determine the distribution of *G. boliviana* in Florida (Fig. 1). The study revealed that *G. boliviana* was well established and spreading on its own in Central and South Florida and no further releases were necessary. However, the beetle was not well established in North-Central and North Florida and additional releases are required (Fig. 2). The results of the study were jointly published in both a scientific and a trade journal.

Beginning in 2004, CPHST Miami and ARS Tallahassee collaborated on a project designed to look at the potential use of *G. boliviana* in Alabama and Georgia to control TSA. Three areas were identified in Alabama and two areas in Georgia to serve as initial release sites to determine if the beetle would over winter in these more northern climates and to possibly serve as field insectaries. Beetles were shipped to state cooperators on a regular basis and released in the selected locations. Prior to release of the beetles, various methods of measuring stand density were tested in order to determine the most effective and time efficient way to evaluate the impact of the beetle. Additionally, patches of tropical soda near the release sites were identified and monitored on a regular basis to determine if the beetles moved from the release sites. The sites in AL and GA differed greatly from central Florida sites. The TSA was limited to isolated areas in small patches often interspersed in wooded areas. In central Florida ranch areas, TSA can form large stands completely covering a multi-acre pasture. Also, TSA is aggressively treated with herbicides in AL and GA, where as in Florida there are many areas that escape management. At each of the five monitoring sites, a 100' transect was established through the highest density of TSA at the location. Along the transect, three 3'x3' patches were permanently established. On each sample date the number of plants crossing the transect line were counted to give an estimate of plant density. In each of the patches the height of the TSA plants were measured, the percentage that TSA composed of the patch recorded, and the number of TSA fruit and *G. boliviana* adults and larvae counted. Beetles were released at the locations for two years and the sites were monitored for two years after the final release.

At all locations TSA disappeared from each of the study sites (Fig. 3 shows the data from a typical site). At the most northern location near Montgomery, AL, the beetles survived through two winters that had periodic snow falls. However, the populations remained very low and eventually

**Figure 1.** The Florida TSA biological team reviewing the protocol for the statewide evaluation of G. bolivana distribution and impact. Pictured (left) Bill Overholt (UFL IFAS), Phellicia Perez (UFL/ER PPQ), Rodrigo Diaz (UF IFAS), John Mass (ARS), Ken Hibbart (DPI), Cathy Marzolf (PPQ FL), Abbie Fox (DPI), Julio Medal (UFL), Stephen Hight (ARS), Scott Weihman (CPHST), and Divina Amalin (UFL/ER PPQ).

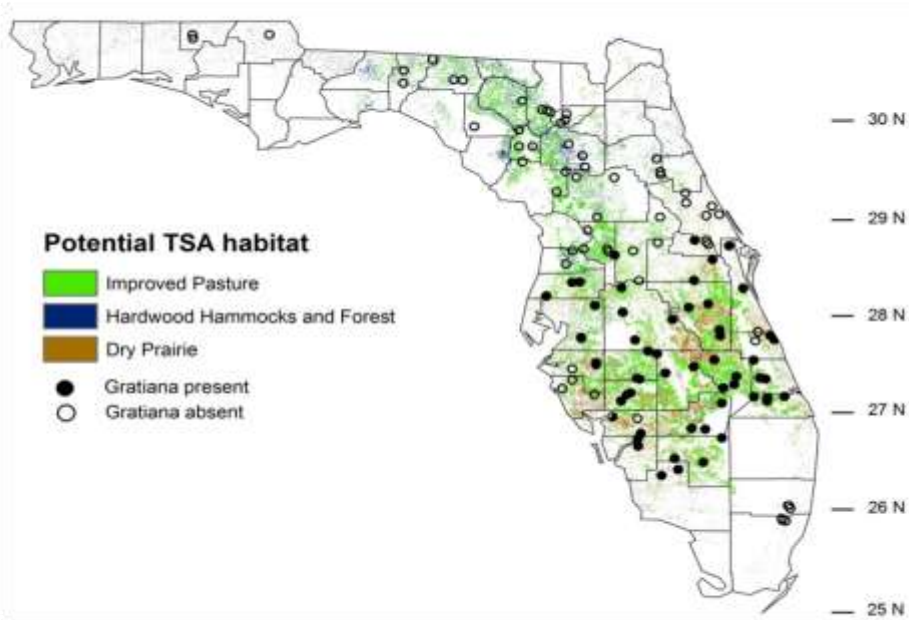


**Figure 2.** Map of sampling locations for *Gratiana boliviana* on its host plant, Tropical Soda Apple in Florida. Open circles represent sites in which *G. boliviana* was absent while solid circles represent sites where *G. boliviana* was present. Suitable habitat is represented as raster cells (green, blue and brown).

disappeared from this location. Although our studies showed that the beetle technically can survive winters in AL and GA, the data generally paralleled those from Florida that indicated the beetle has not established in northern counties. At this point it is unclear if this is because the beetle does less well in cooler climates, the plant does less well in cooler climates, or because of differences in management practices in the north (more aggressive attempts at eradication). However, laboratory studies conducted by University of Florida scientists showed that temperatures at the more northern site in AL were at the biological limits of the beetle. The group is now working on techniques to improve the beetle's chances of establishment in the north, as well as investigating the temperature tolerances and host preferences of a second leaf feeding beetle, *Gratiana graminae*, another potential biological control agent.

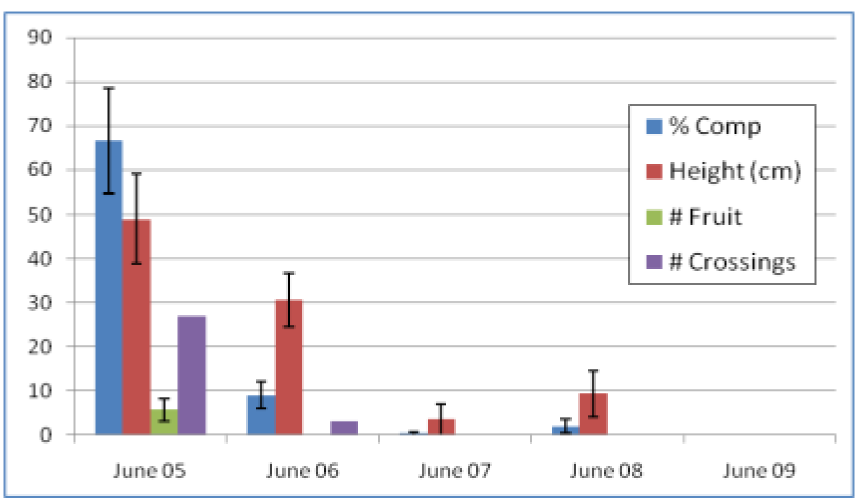


**Figure 3.** The impact of biological control agents (*G. boliviana*) on tropical soda apple in Bainbridge, GA.

## Biological Control of Tropical Soda Apple in East Texas using the Leaf-feeding Beetle, *Gratiana boliviana*

LOCATION: Mission Lab
CPHST STAFF: Dan Flores (lead); Daniel Martinez, Jose Renteria, and Eustorjio Rivas (support)
CHAMPION: Shaharra Usnick and Ron Weeks (WR and ER Biological Control Program Managers)
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Tropical soda apple (TSA), *Solanum viarum* Dunal (Solanaceae), is one of the most destructive invasive plants in America. The weed is originally from South America and first appeared in Florida in 1988. Since then it has advanced to over 1 million acres in Florida and several other states. The fruit is favored by birds, raccoons, coyotes, and cows which distribute the seeds in their manure. Spines cover the leaves and stalks preventing the grazing that might otherwise help control it. The weed serves as a reservoir for diseases and insect pests, invades pastures reducing livestock food, denies cattle access to shaded areas resulting in heat stress, and repeated mowing and spraying costs are significant.

**Figure 1**. A field insectary cage for release of the leaf beetle, *Gratiana boliviana*, was installed in East Texas. Pictured (from left) are Carol Motloch (PPQ), Ricky Thompson (TAMU), and Dan Flores (CPHST).

*Gratiana boliviana* Spaeth (Coleoptera: Chrysomelidae), the TSA tortoise beetle, was identified as a South American insect from Brazil by Dr. Julio Medal in 1994. An unusual defense of the beetle is that the larvae carry their old skin on their back to discourage predators. The females may produce an average of 300 eggs on the leaves or petioles of the TSA plants. Larvae usually feed on the underside of younger leaves. The beetle, which is highly specific for TSA, was first released in Florida in 2003. Since then many thousands of beetles have been successfully released in Florida, Georgia, and Alabama by Amy Roda (USDA APHIS PPQ CPHST - Florida) and Stephen Hight (USDA ARS - Florida).

Scientists from University of Florida, USDA APHIS PPQ CPHST and Eastern Region, USDA ARS - Florida, Alabama Department of Agriculture, and Georgia Department of Agriculture have developed methods for the wide scale, multi-state use of biological control agents as part of an integrated management approach to controlling TSA. Biological control using *G. boliviana* has shown promising results. Beetles established in Florida have shown substantial damage and have significantly reduced the fruit production in TSA plants.

In 1996, a 40-acre field of TSA was detected in Jasper County, Texas, that was accidentally infested through TSA brought in on hay during the drought season. This weed threatens to cause similar losses in Texas to those already experienced in Florida of 90% loss in pasture production. The main goal of this project was to see if the natural enemies would successfully establish in East Texas in order to biologically control TSA infestations.

A plan of action was developed to implement the release of biological control agents as a means to determine their establishment on TSA infestations in East Texas. This work entailed (1) locating an infestation of TSA to serve as a potential field insectary in East Texas, (2) moving *G. boliviana* into Texas and conducting releases of the biological control agents, (3) assessing the establishment of the *G. boliviana* in order to biologically control TSA in East Texas.

A total of 625 adult beetles of *G. boliviana* were released on TSA in East Texas (Figs. 1 and 2) in both summers of 2007 and 2008. For two winters, temperatures reached the low 30s for several days in East Texas, and beetles were observed in the warmer months when temperatures reached the mid 70s. In June 2009, observations made inside the cage and those made by the landowner, indicated that the beetles can survive under East Texas conditions. TSA plants inside the cage and the few plants along the perimeter outside the cage showed significant signs of distress; the leaves were perforated and limp; and some branches were defoliated by the beetles.

The chemical treatment of TSA plants made it difficult to scientifically evaluate and assess the movement and impact of *G. boliviana* as a biological control agent in East Texas outside of the field cage. No other plants have been observed or reported in the surrounding area. Should any be in existence, eradication efforts should be immediately implemented. However, we believe that biological control will offer the best long-term hope for managing TSA populations, especially in areas that may be hard to access. Currently, we await the Technical Advisory Group's response of 2 additional beetles, *Metriona elatior* Klug and *Gratiana graminea* Klug (Chrysomelidae) for potential releases.

**Figure 2.** *Gratiana bolivian*a was shipped from Florida into Texas for the biological control of tropical soda apple.

## Updates on the Light Brown Apple Moth (LBAM) and Beneficial Weevil Projects

LOCATION: Albany Station (Fort Collins Lab)
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CHAMPIONS: Helene Wright (CA SPHD); BLM; CDFA
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Light Brown Apple Moth (LBAM): Rearing technology for the mass production of LBAM (Fig. 1) was optimized and transferred to the mass rearing facility in Moss Landing, CA. The size of the LBAM colony doubled for many months while trays of eggs and diet (Fig. 2) were shipped to initiate an LBAM colony at our Moss Landing facility. Newly hired technicians for Moss Landing were trained in handling LBAM and their rearing procedures.


**Figure 1.** Light brown apple moth adult, courtesy of Todd Gilligan, www.tortricid.net.

LBAM specimens were sent, when requested, to various research institutions and USDA branches in compliance with APHIS quarantine and safety regulations. Institutions which received specimens included: Dr. Norman Barr, USDA APHIS PPQ CPHST, Edinburg, TX; Dr. Robert Koch, University of Minnesota; Dr. Joseph Morse, University of California Riverside; Kira Zhaurova, USDA APHIS PPQ, College Station, TX; Dr. Mark Epstein, California Department of Food and Agriculture (CDFA) Sacramento, CA; and Dr. William Roltsch, CDFA Sacramento, CA.

**Figure 2.** LBAM rearing containers containing diet and developing larvae.

Our cooperation with Dr. William Roltsch of CDFA is an ongoing project on the biological control of LBAM utilizing commercially available and native parasitoids. The project has been successful and is yielding promising results in field tests.

In addition, we are conducting experiments to improve rearing efficiency (Fig. 3). Tests include alteration of diet nutritional qualities, light regimes, egg:diet ratios, and the effect of density on survival of larvae.

**Figure 3.** Egg strips being collected from an adult LBAM cage.

2009 LBAM Presentations:

Poster: Native *Trichogramma* parasitism of the newly introduced light brown apple moth in California. Presented with CDFA, 3rd International Symposium on Biological Control of Arthropods. Christchurch, New Zealand. 8-13 February 2009 (Roltsch W.J., & N.T. Carruthers).

Poster: Effect of Nutrasoy® levels in artificial diet on survival, fecundity and longevity of light brown apple moth *Epiphyas postvittana*. Indianapolis, IN. 12-16 December 2009 (Carruthers N.T., Madieros D., Sims M., & Shelly T.).

Beneficial Root Feeding Weevils: The umbrella project "Development of rearing systems for beneficial root-feeding insects" is comprised of three different sub-projects based on utilizing rearing systems for facilitating biological control of exotic and invasive species:

1. The rearing system for *Hylobius transversovittatus* is fully developed and technology is transferred and utilized in several states and one tribal insectary. Thousands of insects are produced and distributed to customers for the control of purple loosestrife. Although our laboratory colony of this species is terminated, we

still play an important role as a hub for field customers to exchange experiences and ideas associated with rearing this insect in production facilities.

2. The rearing system for *Cyphocleonus achetes* to control knapweeds was also improved (Fig. 4). Nutritional and physical properties of the diet were altered. Stepwise advances were made by testing the effects of different levels of cholesterol, sucrose and cellulose on survival of larvae. In 2009 more than 350 $9^{th}$ and $10^{th}$ generation adults were produced. Our best performing diet yields ~40% survival (Fig. 5). Extensive interest in our rearing system by foreign countries led to changes in the diet formulations, based on world market availability of nutrients.

3. Achievements were made in the use of artificial diets developed in Albany, CA, for facilitating foreign exploration. Our cooperators in Rome from Biotechnology and Biological Control Agency used our diet in field collections for over 20 genera of insects, including a Cerambycid longhorn beetle that is a potential agent for perennial pepperweed. These beetles were collected as larvae, placed on artificial diet, and reared to adulthood.

2009 Weevil Presentation:

Invited presentation of our work was given in November 2009 at the meeting organized by the Department of Interior: Bureau of Land Management, Montana. Dakotas State Office in Billings, MT.

**Figure 4**. Sterilizing eggs of *Cyphocleonus achetes*.

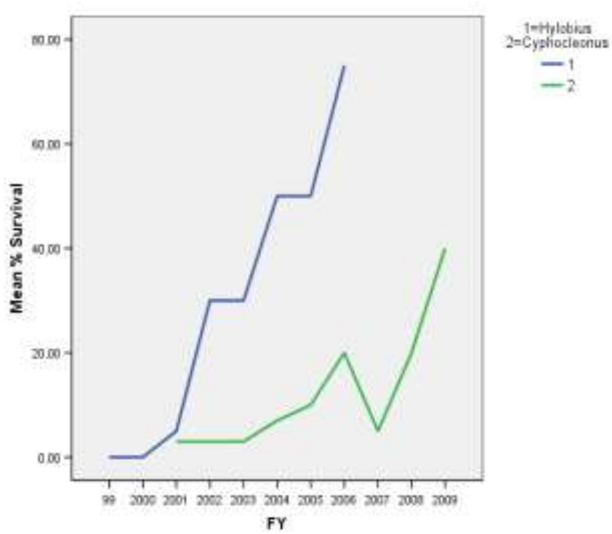


**Figure 5.** Yield improvement of two beneficial weevil species through diet development.

# Evaluation of Recent Isolates of *Metarhizium anisopliae* Against Caged Mormon Crickets on Mini Field Plots in Sidney, Montana, and Logan, Utah, 2009

LOCATION: Phoenix Station (Fort Collins Lab)

CPHST STAFF: Nelson Foster (Lead); Larry Jech; Chris Reuter; Lonnie Black

CHAMPIONS: Charlie Brown (Nat. GH/MC Program Manager); Roeland Elliston (WR Program Manager); Robert King (Utah and Nevada SPHD)

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Rangeland grasshopper treatments in USDA sponsored programs rely on traditional insecticide sprays or baits. Environmentally sensitive areas encountered within potential program areas commonly preclude the use of traditional insecticides. The number of sensitive areas is increasing and in areas of grasshopper and/or Mormon cricket infestations, these situations can complicate or prevent much needed local or area-wide grasshopper treatments. Treatments allowed in such areas could be extremely important to both local and area wide control efforts.

The development of non-chemical entomopathogens has long been desired as an alternative to traditional pesticides for control and management of grasshoppers and Mormon crickets on rangeland in the United States. While *Paranosema locustae*, a protozoan parasite of grasshoppers and *Beauveria bassiana*, a fungus with a broad insect host range have been registered for use against these pests, neither one has gained wide acceptance nor use. The fungus *Metarhizium acridum,* which has activity specific to Orthopterans has been found in Australia, Africa, South America and Mexico is registered for use in several countries. However, it has not been found in the US nor have foreign strains been registered for use in the US.

With the end goal of developing alternative treatments to traditional insecticides, scientists from CPHST and ARS have been collaborating since 2003 to better understand the parameters under which the native fungi *Beauveria bassiana* and *Metarhizium anisopliae* show useful activity against grasshoppers or Mormon crickets on rangeland. In 2007, CPHST scientists began collaborating with Utah State University scientists toward the same goal. This group of scientists together with the USDA APHIS PPQ Western Region efforts to supply soil samples with potential pathogens, works as a team to specifically develop usable, newly discovered fungal isolates of broad insect host range for rangeland grasshopper and Mormon cricket treatments and more importantly, the development of *Metarhizium acridum* when found in the US.

The USDA APHIS PPQ Western Region provides soil samples from the western states taken during routine field surveys for grasshoppers and Mormon crickets. Utah State University processes the samples and screens them for new isolates of *Beauveria bassiana, Metarhizium anisopliae* or *Metarhizium acridum* (yet to be found). Both Utah State University and ARS scientists then evaluate the strains in the lab for potential activity against the target pests and for growth/reproductive potential for mass production. The group then selects the most promising isolates which are then produced to field testable amounts by ARS, USU or both. CPHST, ARS and USU scientists then work together in the field to evaluate the isolates against Mormon crickets and/or grasshoppers. Isolates are sprayed as liquid treatments or applied as solid baits depending on the study design. Sprays are applied to hun-

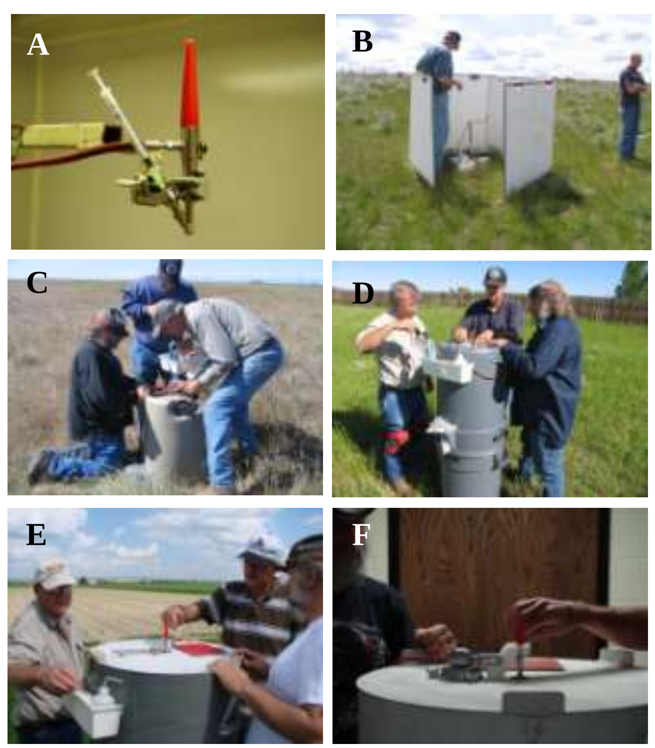


**Figure 1.** Developed in late 1980's as an alternative to traditional topical application laboratory studies (Foster et al. 1991), the air brush (Paasch Type H with 75 regulator) modified with a customized syringe needle (A) for liquid injection can produce droplets that simulate aerially applied sprays with known realistic field application doses. The system was made portable and used in the field originally with windbreaks to minimize spray drift (B). It evolved into a small portable prototype enclosed chamber (C) and larger more functional chamber (D). Today, the system has been adapted to a non-static producing paperboard chamber (E, F) and is termed Field Aerial Application Spray Simulation Tower Technique (FAASSTT).

dreds of mini plots by CPHST scientists using the Field Aerial Application Spray Simulation Tower Technique (FAASSTT) (Fig. 1), a system developed by CPHST to simulate aerial applied sprays at realistic field rates. After treatment of small plots, test insects are caged on the treated areas and monitored daily for mortality until the end of the study. (Fig. 2) Studies usually include 6 replications

**Figure 2.** Field cages arranged on treated mini-plots. Cages were removed during spray treatment, then replaced and stocked with a single immature Mormon cricket. Paper plates were placed on top of cages to protect the spray from a rain storm.

of at least 12 individual mini plots and cages per replicate for each treatment in the study. Two replications of each treatment in each study are typically removed from the field after two days and brought into the lab where they are held at optimal temperature for fungal growth to confirm that a lethal dose was delivered in the field. During the studies, thermal surrogates for basking insects, in the form of wire thermocouples inserted into small, soy-sauce-filled tubes and attached to temperature recording devices, are used to determine the temperatures the test insects and the fungi infecting them experience during normal behavior in the field and to determine the amount of growth the fungi could be expected to produce in response to the observed “body temperatures”. This information is eventually used to predict when onset of mortality could be expected to occur under the conditions of the specific study and is critical to understanding environmental limitations to these fungi.

Two major studies of this type were conducted in 2009. Cooperating (CPHST, ARS, USU) scientists, using FAASSTT, applied candidate formulations of previously collected domestic fungi, pathogenic for Mormon cricket, to 1,104 mini rangeland plots (684 in Sidney, Montana and 420 in Logan, Utah). The studies were expected to; (1) compare the activity of three domestic strains of *Metarhizium anisopliae* against Mormon cricket; (2) provide initial dose ranges for further evaluation, and (3) to evaluate persistence of one of the strains. Each study lasted about 50 days.

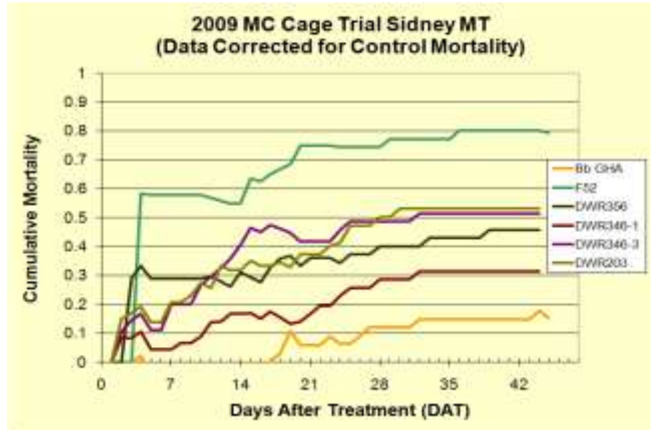


**Figure 3.** Average percent Mormon cricket field cage mortality, Sidney, MT.

In Sidney, MT, *Metarhizium anisopliae* isolates F52 high rate, DWR356 low rate, DWR346 low and high rates, DWR203 low rate and the *Beauveria bassiana* strain GHA high rate were studied. In our study, F52 performed the best and reached 85% mortality by 37 DAT. The other treatments produced substantially lower mortalities while *B. bassiana* produced the lowest mortality. Important to note is the dose rank order result of DWR346 (Fig. 3).

The associated lab incubation study demonstrated that a sufficient dose to produce a great degree of infection and mortality was applied in the field, with all treatments producing 90-100% mortality by 14 DAT and two producing 100% mortality within a week (Fig. 4). These laboratory incubations demonstrated efficacy in the absence of adverse, outdoor, environmental effects and should be contrasted with efficacies shown in Fig. 3.

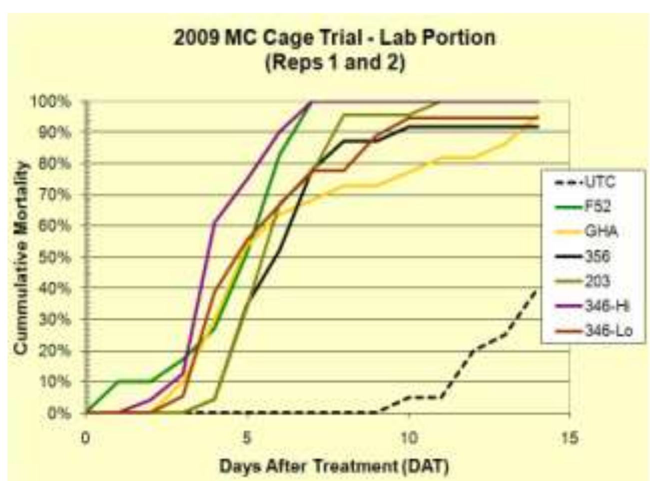


**Figure 4.** Laboratory confirmation of the efficacy of the field applied fungi, Sidney, MT (UTC = untreated control).

In Logan, UT, the *Metarhizium* isolates DWR203, F52, DWR356, and DWR346 all applied at the high rate were studied. In this study, DWR346 and DWR203 produced the highest mortalities respectively. DWR346 approached 70% mortality at 30 DAT (Fig. 5). The lab incubation study showed that doses sufficient to result in mortality were administered in the field with similar results to those seen in Sidney, MT. Two strains produced 100% mortality by day four and all strains produced 100% mortality within 8 days after treatment (Fig. 6).

The meteorological conditions at the two study locations help explain some of the differences in overall results. Further evaluation of the data is continuing and further cooperative studies are planned. The long term potential of the overall cooperative effort is reflected in the number of new isolates that have been identified by USU. To date this number exceeds 1,500. Since each isolate may operate under its own and sometimes unique temperature parameters, the aim is to find one compatible with the environs that US rangeland grasshoppers and Mormon crickets inhabit. It is important to note that this large scale discovery effort may produce isolates that can be very useful against other non-related pests, current or as yet unforeseen.

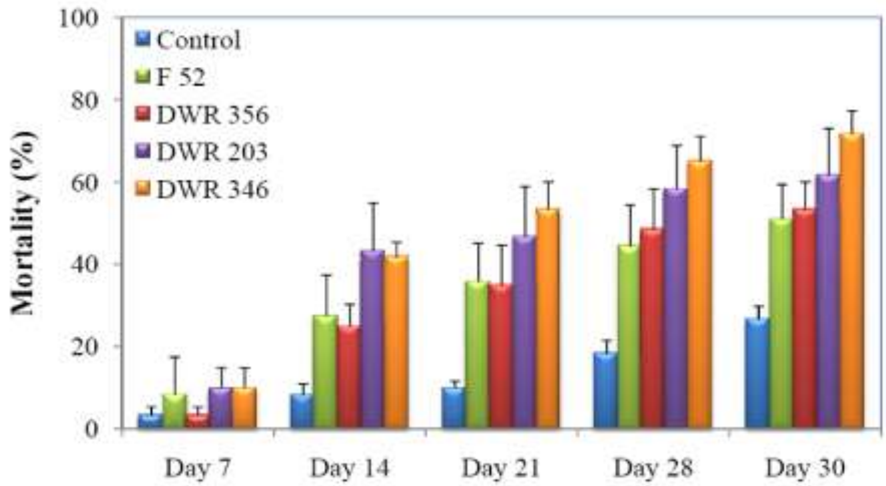


**Figure 5.** Average percent Mormon cricket field cage mortality, Logan, UT.

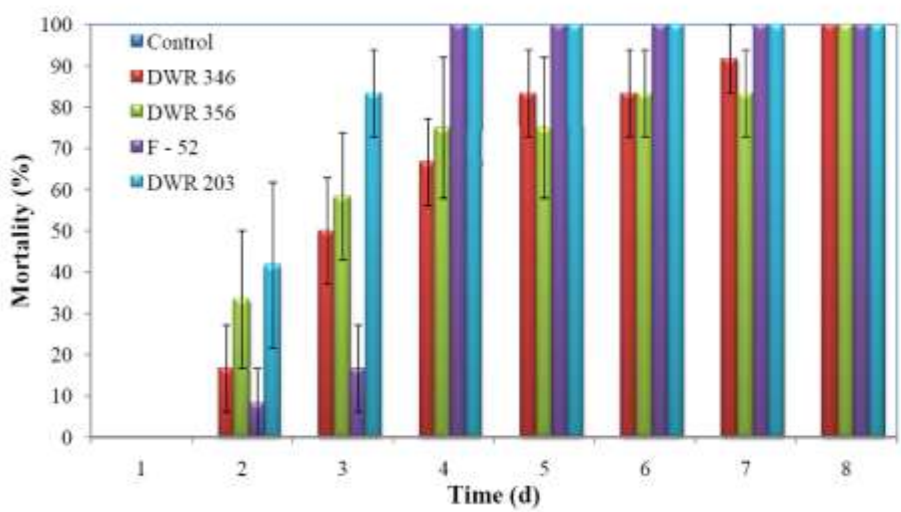


**Figure 6.** Laboratory confirmation of the efficacy of the field applied fungi, Logan, UT.

# Biological Control of Emerald Ash Borer (*Agrilus planipennis*)

LOCATION: Otis Lab
CPHST STAFF: Juli Gould (Lead), Tracy Ayer, David Williams, Ivich Fraser, and Vic Mastro.
CHAMPIONS: Philip Bell (ER); Paul Chaloux (EDP)
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The Pest: The emerald ash borer (EAB), an exotic invasive wood-boring beetle (Fig. 1) native to Asia, is threatening ash trees throughout North America. EAB was first detected in Michigan in 2002, although it likely arrived more than ten years earlier in solid wood packing materials. As of December 2009, EAB has been detected in thirteen states (Indiana, Illinois, Maryland, Michigan, Missouri, Ohio, Pennsylvania, Virginia, Wisconsin, West Virginia, Kentucky, New York, and Minnesota) and the Canadian provinces of Ontario and Quebec. Forest inventories report 8 billion ash trees on U.S. timberlands, of which 693 million occur in Michigan. It is estimated that over 70 million of Michigan's ash trees have already succumbed to EAB. Treatment options are limited and relatively few native natural enemies (parasitoids, predators and pathogens) attack EAB.

**Figure 1.** Adult EAB (Tracy Ayer, USDA, APHIS, PPQ).

Biological Control: Because EAB is from northeastern Asia, U.S. and Chinese scientists have been searching for EAB and its natural enemies in that region since 2003. Several EAB parasitoids were discovered in collaboration with scientists at the Chinese Academy of Forestry. USDA scientists are currently evaluating two larval parasitoids and one egg parasitoid from China. CPHST scientists are conducting research on one of the larval parasitoids, *Spathius agrili*, which was found parasitizing up to 90 percent of EAB larvae in ash trees in China. Female *Spathius* parasitize EAB larvae by drilling through the bark (Fig. 2A) and laying up to 20 eggs on its host. The hatching parasitoid larvae feed and develop on the EAB larva (Fig. 2B), resulting it its death. The cycle is repeated 3-4 times each summer and fall. *Spathuis* overwinter as pupae inside cocoons under the bark of ash trees and emerge as adults in the summer.

Project Status: Laboratory methods for continuous rearing of *S. agrili* have been developed. The specificity of this parasitoid was studied extensively on native beetles and other insects. In laboratory no-choice assays, *S. agrili* parasitized a few species of wood borers in the genus *Agrilus*, however, parasitism rates were significantly lower than when parasitizing EAB. The preference of these parasitoids for EAB and ash habitats was further confirmed through surveys in China and olfactometer tests.

An Environmental Assessment outlining the benefits of releasing these three parasitoids without posing a significant risk to native wood borers or their environs was prepared and published in the Federal Register for a 60-day comment period. Permit applications for field release of *S. agrili* and the two other parasitoids were reviewed by international, national and state scientists and regulators, and permits were granted in July of 2007. Small numbers of each species were released in Michigan in the fall of 2007 and CPHST scientists confirmed *S. agrili* successfully reproduced in Michigan field sites and survived the winter in spring 2008. In FY2009 a major focus was placed on transferring the technology for rearing *S. agrili* to the mass rearing facility in Brighton, MI. Over 8,000 parasitoids reared by the new facility, as well as at the Otis lab, were released in 2009. Releases of the three species will continue while scientists further determine how these natural enemies can best be used to help suppress EAB populations. Scientists also continued to survey for and investigate the potential of additional EAB natural enemies that might be used in a biological control program. During 2009, new species of *Spathius* and *Tetrastichus* were discovered in South Korea and Russia.


**Figure 2.** *Spathius agrili* .

**2A.** *Spathius agrili* ovipositing on EAB through ash bark ;

**2B.** *Spathius agrili* larvae consuming EAB host.

(Photos: Dr. Yang Zhong-qi, Chinese Academy of Forestry).

## Biological Control of Winter Moth

LOCATION: Otis Lab
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In a cooperative program with the University of Massachusetts (Dr. Joseph Elkinton), the Otis Laboratory has been developing a biological control program for the winter moth, *Operophtera brumata*. This effort has been ongoing for approximately 5 years. The Laboratory has also been involved with optimizing the trap and pheromone lure combination for winter moth to enhance our ability to conduct surveys.

The winter moth was discovered infesting trees in eastern Massachusetts approximately 9 years ago. It is a very early spring defoliator of numerous species of deciduous trees and shrubs. In addition to damage on urban and forest trees, it can also cause significant damage on crops such as blueberries and apples. Adults are active in November and/or December. Males are active fliers, even at low temperatures, while females are flightless and virtually wingless. The females, emerging from pupation sites in the soil, crawl up host trees where they mate and deposit their eggs. Eggs hatch in the very early spring and begin feeding by either mining the unexpanded buds of plants or attacking newly expanding leaves. In heavy infestations, leaves are fully consumed before they ever get a chance to expand fully. Most hardwood trees will re-foliate, however, repeated defoliation by winter moth or subsequent defoliation by other defoliators (gypsy moth, forest and eastern tent caterpillars, etc.) will result in mortality. Smaller bushes are even more susceptible and can be killed by a single defoliation.

Earlier in the century, winter moth was introduced into Nova Scotia where it caused extensive defoliation and damage. In 1954, authorities began releasing a biocontrol agent imported from Europe, *Cyzenis albicans*, a parasitic fly. It took 5 years (1959) before they were able to recover any *C. albicans* and demonstrate establishment, but by 1962, moth populations had decreased and little defoliation was apparent. Since that time, winter moth populations in Nova Scotia have remained low. The winter moth was also accidently introduced into Victoria, British Columbia, and the northwestern USA in the 1970s. Subsequent releases of *C. albicans* have also provided good control there.

At the Otis laboratory we have evaluated various trap and lure combinations for the winter moth and arrived at a system that is highly efficient and easy to use by surveyors. Also, we were able to develop a rearing technique for winter moth, as well as a system for collecting and overwintering parasites from British Columbia. Unfortunately to date establishment of the fly has not been confirmed in MA. In 2009, the FS increased collections of *Cyzenis* in British Columbia, and several thousand of these parasitic flies now await release in 2010.

## Biological Control of *Sirex noctilio* in North America by *Beddingia siricidicola*

LOCATION: Otis Lab
CPHST STAFF: David Williams (Lead), Carrie Crook
CHAMPIONS: Robyn Rose, Leon Bunce, Yvonne DeMarino, Coanne O'Hern
CONTACT: Dave Williams (david.w.williams@aphis.usda.gov; 508-563-9303 ext. 217)

The European woodwasp, *Sirex noctilio,* was discovered in Oswego County, New York, in the fall of 2004. Since then surveys have found *Sirex* throughout central and western New York as well as Pennsylvania, Michigan, Vermont, Ohio, and Ontario, Canada. The woodwasp is apparently not under effective natural control and is already distributed over a wide area in North America. Because it kills relatively healthy trees, *Sirex* poses a serious threat to pine forests and plantations in the United States and Canada if it is not managed. *Sirex* presents perhaps the greatest risk to the largely unmanaged pine monocultures in the southeastern United States.

The most effective natural enemy of *Sirex* woodwasp is a parasitic nematode, *Beddingia siricidicola*. This nematode has been used successfully as a biological control agent in management programs throughout the southern hemisphere where *Sirex* woodwasp has invaded. The nematode's unique life history facilitates its use as a management tool. Dependent upon physical conditions in its microhabitat, it can develop into either of two forms. The fungus-feeding form feeds on the *Sirex* symbiotic fungus, *Amylostereum areolatum*, as it builds populations inside a tree attacked by woodwasps. The parasitic form develops in proximity to *S. noctilio* larvae and attacks them, ultimately

**Figure 1.** Mass rearing nematodes and fungus on brown rice and wheat berry.

sterilizing the woodwasp females. The fungus-feeding form can be mass-reared easily in the laboratory for field release (Fig. 1).

Our biological control program uses the highly pathogenic "Kamona strain" of *B. siricidicola* obtained from Ecogrow, the licensed nematode producer in Australia. However, as early as 2006, we found a nematode already infecting woodwasps in our study plots in New York. This "native strain" is similar in appearance to the Kamona strain and has been identified as *B. siricidicola*. We speculate that it entered North America along with *S. noctilio*. It is known to be present within at least an 80-km radius of Syracuse, New York. The Canadian Forest Service reported a similar strain in 2008 and identified it as *B. siricidicola* using DNA analysis. Clearly, the presence of a strain of the species that we are releasing poses a challenge to our evaluation of the Kamona strain as a biological control agent and necessitates the development of powerful molecular tools for discriminating nematode strains.

We have conducted four controlled releases in the fall during the 2006/7, 2007/8, 2008/9, and 2009/10 seasons (Figs. 2 and 3). The goals of the studies were to test the Australian inoculation method, to assess the establishment of Australian nematodes in American pines, and to evaluate overwintering survival of the exotic nematodes under North American conditions. The releases were "controlled" so that no nematodes could escape into the environment. Trees were inoculated in the fall, and billet samples were collected in winter for rearing of *Sirex* adults in the lab. All remaining tree materials were destroyed before insect emergence in the spring. The releases were terminated primarily because of concern as to possible impacts of the nematode on non-target native siricids.

Possible effects of the biological control program on non-target native siricid species continue to be of concern. Eastern North America has three native siricid species that use *Pinus* species as hosts: *Sirex edwardsii*, *S. nigricornis,* and *Urocerus cressoni*. The fungal symbiont of these species is the key to understanding their susceptibility to *B. siricidicola.* Two species of *Amylostereum* fungus are commonly associated with siricids worldwide. *Amylostereum chailletii* is native to North America, whereas *A. areolatum* is native to Europe. The nematode survives and reproduces only on *A. areolatum.* Thus, siricids that feed on *A. chailletii* have a refuge from nematode parasitism. Because of their North American origin, the three pine-feeding native siricids are likely to live on *A. chailletii,* and thus, not to be exposed to the nematode. However, a recent study found that *S. edwardsii* can feed on *A. areolatum,* and thus, may potentially be infected by *B. siricidicola*. *Sirex nigricornis* and *U. cressoni* both feed on *A. chailletii* and thus are not susceptible to the nematode. Another siricid of non-target concern in eastern North America is *Xeris spectrum*. It does not have a fungal symbiont and may feed on either *Amylostereum* species, thus rendering it potentially susceptible to the nematode. Because impacts on some of the native siricids cannot be ruled out, the decision to release *B. siricidicola* may ultimately rest on a cost-benefit analysis of possible impacts on non-target woodwasp species versus the health of our forest resources.

**Figure 2.** Punch hammer and pine bole with inoculation holes at 6 inch spacing.

**Figure 3.** Inoculating nematodes into a tree.

## Florida and Offshore Biological Control Initiative against Invasive Species

LOCATION: Miami Lab
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CHAMPIONS: Ron Weeks (ER Regional Program Manager)
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South Florida is particularly vulnerable to invasive pests because of the high volume of agricultural imports entering this area and its proximity to the Caribbean. Since 1971 exotic insects have been arriving and becoming established on Florida's shore at a rate of about 10 species per year. The Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FL DACS-DPI) indicated that approximately 150 species of exotic arthropods established in Florida from 1986-2000. Of those 150 species introduced into Florida, 57% were first found in Monroe, Miami-Dade, Broward, and Palm Beach Counties. Many of these invasive pests cause extensive economic damage or pose a significant threat to the agriculture of Florida and the United States. For example, the Asian citrus psyllid transmits citrus greening disease (or 'huanglongbing') and threatens Florida's $9 billion citrus industry. To confront this problem, Eastern Region PPQ funds a University of Florida cooperative agreement with University of Florida IFAS Tropical Research and Education Center to produce and evaluate natural enemies for managing these invasive pests. Aggressively perusing biological control programs in South Florida helps slow the spread of these invasive species throughout Florida and into other regions of the US. The personnel (one post-doctoral research assistant and two full time technicians) funded by the cooperative agreement are co-located with the CPHST Miami Biological Control Unit at the ARS Subtropical Horticulture Research Station (SHRS), Miami, FL. The close, on-site association, particularly with CPHST off-shore programs, enhances the Miami project by a direct transfer of the latest developments in biological control technology to the south Florida specific programs. With the expertise and basic resources in place, this program has been able to provide a rapid response to newly detected invasive pest species by evaluating the impact of the pest, determining the effect of local natural enemies, and rearing and releasing biological control agents. In 2009, the Miami Biological Control Unit worked closely with this program on two important pests: the croton scale (*Phalacrococcus howertoni*), a new pest first described in Florida, and the cactus mealybug (*Hypogeococcus pungens)*, an invasive pest devastating endangered cactus species in Puerto Rico.

### Cactus Mealybug

The cactus mealybug is a pest of major concern in Puerto Rico attacking 13 species of cacti, 3 of which are endemic and 2 are near extinction (Fig. 1). It could threaten similar species in the Western U.S. The mealybug is also known to occur throughout Florida since 1984, but it has a very limited host range and appears not to be a significant pest.

| Counties | # sites visited | # positive sites | # of mealy-bugs | Percent parasitism |
|---|---|---|---|---|
| Brevard | 4 | 0 | | |
| Broward | 5 | 1 | 24 | 45.83% |
| Charlotte | 1 | 0 | | |
| Clay | 1 | 0 | | |
| Flagler | 1 | 0 | | |
| Highlands | 2 | 1 | 5 | 0.8% |
| Hillsborough | 1 | 0 | | |
| Leon | 1 | 0 | | |
| Lee | 5 | 2 | 7 and 75 | 16-42.85% |
| Manatee | 1 | 0 | | |
| MIiami-Dade | 9 | 1 | 1 | 0% |
| Orange | 5 | 0 | | |
| Pinellas | 8 | 2 | 41 and 104 | 0-0.96% |
| Polk | 3 | 1 | 1 | 0% |
| Seminole | 3 | 0 | | |
| St. Lucie | 2 | 0 | | |
| Volusia | 6 | 1 | 23 | 37.8% |

**Table 1.** The number of cactus mealybug, *Hypogeococcus pungens* and percent parasitism found in 17 Florida counties surveyed summer of 2009.

In 2009, surveys for natural enemies of *H. pungens* were conducted in sites where the pest was previously collected. A total of 58 locations in 17 counties were surveyed. Out of the 58 locations, only 9 locations had *H. pungens* infestations (Table 1), 6 of which contained parasitized *H. pungens.* The parasitized mealybugs were encapsulated to identify the parasitoids and determine the impact of these natural enemies. Percent parasitism ranged from 0 to over 40% (Table 1). Parasitoids that emerged were submitted for identification to SEL, USDA-ARS, Beltsville, Maryland. The multiple predatory beetles and the primary parasitoid, *Gyranusoidea pseudococci,* that were found widely distributed across Florida are likely proving good biological control of the pest. The Florida natural enemies may work as classical biological control agents against the cactus mealybug in Puerto Rico.

### Croton scale

Croton scale, *Phalacrococcus howertoni*, a new species described from Florida, was collected on April 9, 2008 at a nursery in Marathon, FL (Monroe County), on a croton plant. After the initial find, it was also found on other plants such as guava, gumbo limbo, lignum vitae, mysore fig, strangler fig, mango, island marlberry, firebush, wild coffee and West Indian satinwood. The economic importance of this pest is unknown at this time but its detection on more than 70 different host plant species is alarming. The scale has been collected from 21 Florida counties thus far with establishments in natural environments in 5 counties. In 2009, abundance of the Croton scale and its natural enemies was examined monthly at 6 residential croton plantings located in south Florida. The survey revealed a wide array of natural enemies attacking the croton scale. Two species of parasitoids (*Metaphycus flavus* and *Signiphora bifasciata)*, 3 species of coccinellids (*Exochomus* sp., *Chilocorus* sp. and *Azya orbigera*), and 1 species of predatory Lepidoptera (*Laetilla coccidivora*) were collected. One of the identified parasitoids, *Metaphycus flavus*, is known to be a widespread parasitoid of many different species of soft scales; whereas the other parasitoid, *Signiphora bifasciata*, is probably a hyperparasitoid. The most commonly found predator, *Azya orbigera*, was tested in the laboratory. A very high consumption rate was found for both the adults and larvae, indicating the potential of this species as an effective biological control agent of croton scale. The populations of the scale and their natural enemies will be assessed every month until summer 2010.

**Figure 1:** Dr Alejandro Segarra Carmona (University of Puerto Rico) and Dr. Ron Weeks (ER PPQ Program Manager) examine endangered cactus plants being devastated by the cactus mealybug in Puerto Rico.

## Biological Control of Invasive Mealybugs

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Passion vine mealybug: The proximity of the Caribbean Island nations to the United States makes the United States especially vulnerable to the entry of exotic pests that have established in this region. With expanding trade and tourism, other safeguarding strategies are needed to supplement the historic reliance on port-of-entry inspections. Although unfortunate, the presence of invasive pests in the Caribbean and Puerto Rico provides an opportunity to develop methods to recognized field symptoms of the pest as well as survey and control strategies, such as biological control. One pest known to be established in Trinidad and Puerto Rico is the passion vine mealybug, *Planococcus minor,* a pest of over 250 plants including citrus, corn, grape, potato and soybeans. A recent pest risk assessment concluded that the likelihood of this pest becoming established in the USA is high and the consequences of its establishment would be severe (Venette and Davis 2004). From 1985-2008, the pest was intercepted 698 times in the baggage and on commodities coming from the Caribbean. Although present throughout the Caribbean since the early 1990s, this mealybug has not yet developed into a major regional pest. However, because of the mealybug's wide host range concerns about it establishing in the USA continue. CPHST Miami worked with scientists from Florida Agriculture and Mechanical University to design and implement field experiments to survey for *P. minor* natural enemies and determine the impact of the pest in Trinidad. A pure *P. minor* colony was established in the laboratory and used to create infestations on potatoes. Once placed in the field, the potatoes served as tools to identify the natural enemy community responsible for managing *P. minor* populations. This novel technique to monitor for natural enemies proved to be very successful and revealed a large natural enemy complex, including several species of parasitoids, ladybird beetles, predatory flies and hemipterans. Two of the parasitoids collected, *Leptomastix dactylopii* Howard and *Coccidoxenoides perminutus* Girault, have been used successfully in the biological control of a very similar mealybug species, *Planococcus citri* (citrus mealybug). Both of these parasitoids are found in the U.S. and are commercially available.

In 2009, the CPHST Miami Biological Control Unit collaborated with University of Florida, FAMU and ARS (Puerto Rico) on a T-STAR grant entitled "Investigating bio-ecological factors influencing infestation by the passionvine mealybug, *Planococcus minor* (Maskell) (Hemiptera: Pseudococcidae), a potential threat to the U.S." Puerto Rico is known to have both the citrus mealybug and the passion vine mealybug and neither species is reported to be a pest. Having both mealybug species established in Puerto Rico provided an opportunity to confirm that the parasitoids identified attacking *P. minor* in Trinidad in fact attack and provide control of this species when *P. citri* is present. Because the citrus mealybug and passion vine mealybug are morphologically identical, passionvine mealybug pheromone lures produced by University of California cooperators were placed in coffee, citrus, mango, annona and cacao plantations in order to find locations with predominately *P. minor* populations (Fig. 1). Citrus mealybug pheromone traps were also used to find *P. citri* populations. All locations sampled contained both mealybug species. Adult fe-

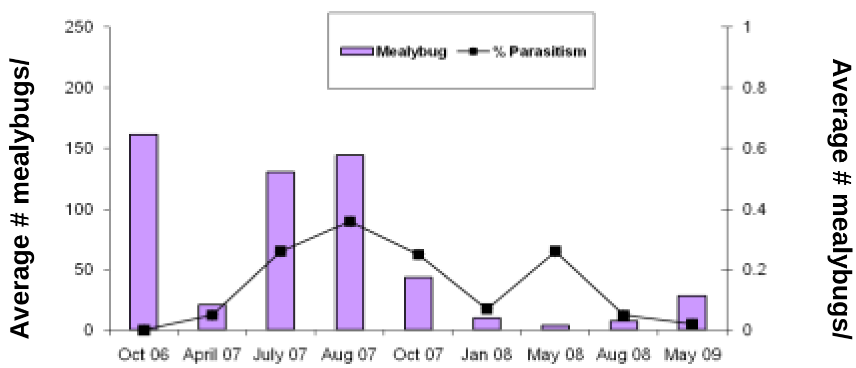
**Figure 1** USDA ARS scientists Dr. David Jenkins worked with CPHST scientist Dr. Amy Roda (A) in Puerto Rico to place pheromone traps in the field to locate populations of passionvine mealybugs collected from plant material like cacao pods (B) such as Dr. Ron Weeks, ER PPQ examines (C).

males were collected from locations with the highest number of passionvine mealybugs caught on the traps. They were placed individually on sprouted potatoes and held in the laboratory. Only the colonies identified as passion vine mealybug were kept for future experiments. The colonies were then used to infest potatoes that were then placed in the field to collect natural enemies as was done in Trinidad. Data from these experiments is still being collected and processed. The group also is developing protocols for deploying the pheromone traps in areas where the passion vine mealybug is not known to occur.

Pink hibiscus mealybug: An example of how methods developed in an off-shore safeguarding program provided an immediate domestic response was seen with the recent invasion of the pink hibiscus mealybug, *Maconellicoccus hirsutus* (Green) (Hemiptera: Pseudococcidae), in Louisiana. In October 2006, pink hibiscus mealybug, a pest of more than 250 different plants, was detected in New Orleans. Within two weeks, two parasitoids, *Gyranusoidea indica* and *Anagyrus kamali*, were released. Such a rapid response was possible because the effective natural enemies had been identified and rearing methods developed off-shore in the Caribbean. Prior to releasing the two wasp species, seven study sites were established and the numbers of mealybugs found on hibiscus growing terminals were counted. After the release of the natural enemies, these sites were sampled periodically to determine if the natural enemies were reducing the mealybug populations. Initially, there was a dramatic reduction in pink hibiscus mealybug numbers (Fig. 2). However, in the summer following the first release, pink hibiscus mealybug numbers increased nearly to levels seen when the pest was first detected. In discussions with the Louisiana Dept. of Agriculture and PPQ LA it was decided the initial decline was most likely due to the normal seasonal decline in mealybug populations during the cooler winter months rather than the action of the parasitoids. However, the data showed that the parasitoids were still present in the study sites and no further releases were made. The study sites were continuously monitored through May 2009. As had occurred at other infestation and releases sites (Caribbean, CA, FL), the parasitoids were shown to provide good control of PHM within one year of their initial release. By May 2009 PHM population numbers had been reduced by > 83% and only 2 of the 6 remaining sites had mealybugs.

Both *G. indica* and *A. kamali* became established in New Orleans; however, additional studies (encapsulation of 3rd instar -young adult PHM females) showed that *G. indica* was recovered more frequently than *A. kamali* (83% of the identified exotic parasitoids vs. 17%, respectively) (Fig. 3). This is in contrast to results from warmer areas where *A. kamali* is typically found in higher numbers and demonstrates the utility of releasing both species to manage PHM infestations.

**Figure 2.** Average number of pink hibiscus mealybug (2nd-adult stages) per hibiscus terminals collected from 7 sites in Louisiana (± SEM). October 2007 mealybug numbers were 73% lower than originally found in October 2006 (83% reduction Oct 06 –May 09). May 2009 only 2 of the 6 remaining sites had mealybugs. The parasitism rate includes all primary, hyperparasitoids and mummies (parasitized mealybugs where the parasitoid did not emerge).



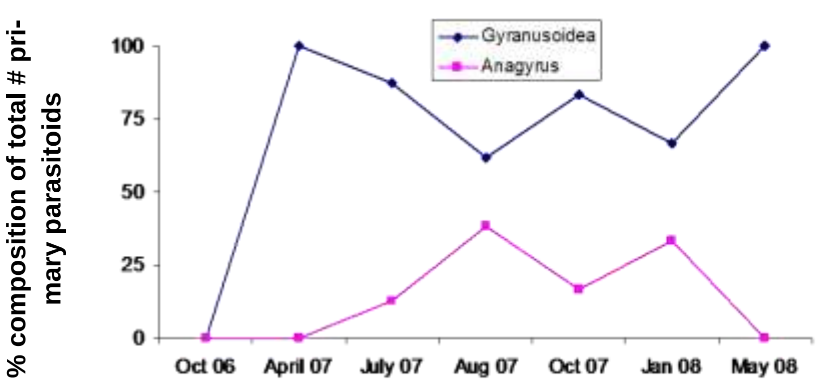




**Figure 3.** *Gyranusoidea indica* composed the greatest percentage of the primary parasitoids collected at the seven sites compared with *Anagyrus kamali*. The numbers of *A. kamali* increased through Aug. 07. Studies in California indicated that *G. indica* is more cold tolerant than *A. kamali*. The prevalence of *G. indica* in the cooler months of April, Oct and Jan also suggest cold tolerance. Laboratory experiments are under way to verify these patterns. No primary parasitoids emerged from the Aug 08 or May 09 encapsulations. The parasitized mealybugs will be dissected for possible parasitoid species determination.

# Biological Control of the Imported Fire Ant Using Phorid Flies: Cooperative Rearing and Release Project, 2009 (*Pseudacteon tricuspis, P. curvatus,* and *P. obtusus*)

LOCATION: Gulfport Lab
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In a USDA-APHIS survey in 2000, seven southern states ranked IFA as a top priority target organism for biological control. Phorid flies (*Pseudacteon spp*.) from South America are promising biological control agents because they are relatively specific to IFA, are active throughout most of the year, and through suppression of fire ant activity, may allow native ants to compete with IFA for food and territory. In South America there more than 15 species or biotypes of phorid flies that impact IFA, and thus are candidates for rearing and release in the U.S.

USDA, APHIS, PPQ began funding a cooperative project with USDA, ARS and FL-DOACS-DPI in 2001 to mass-rear and release phorid fly species as they became permitted. Preliminary research, host range testing, release permits and the development of rearing techniques have largely been under the direction of USDA, ARS Gainesville, FL. Thus far, three species have been approved for release, with others under development. The goal is to release 5-6 species of phorid flies throughout IFA infested states. ARS will continue to evaluate and transfer rearing techniques to the rearing facility as the new species are ready for mass rearing. Mass rearing of flies is being conducted by the FL-DOACS Division of Plant Industries (DPI), in Gainesville, FL, with funding provided by APHIS-PPQ HQ, WR, ER and CPHST. The CPHST biological technician position assigned to the rearing facility was transferred to the cooperative agreement with DPI when the position was vacated in early 2008. The position was refilled by one of the FL-DPI qualified and experienced technicians as a promotional opportunity. This position continues to coordinate the shipment of phorid flies to field cooperators as well as assist in production duties and perform methods development experiments to improve rearing techniques or solve problems as needed. CPHST Gulfport prioritizes release sites, lines up and train state cooperators to make releases and track establishment.

Since 2002, two species of *Pseudacteon* sp. flies have been released at multiple sites in all IFA quarantined states in the contiguous southeastern states and Puerto Rico (no releases in NM and only one species released in CA) and field releases with a third species began in 2008. From 2002 through 2009 there have been 105 field releases of phorid flies and more than 933,000 potential flies released. Of these 105 releases, 64 were *P. tricuspis,* 34 were *P. curvatus* and 7 were *P. obtusus*. The average number of potential flies per release is about 10,000 flies. In 2008, the changing economy had an impact on our cooperators' abilities to conduct releases, and due to lack of resources in many states, the number of releases in 2008 dropped to only 8 (Table 1). In 2009, we were able to increase our releases from 2008 back to 12.

In 2009, 4 attack (rearing) boxes were online producing the first species of fly, *P. tricuspis*, 7 boxes were being used to produce the second species, *P. curvatus* (Formosan biotype), and 4 boxes were producing a third species of fly, *P. obtusus* (see Table 1 for production numbers). A total of 16 boxes are available for rearing, however 1-2 boxes are maintained for research purposes to improve rearing techniques and develop techniques for new species.

Success of the program was originally measured by successful overwintering of fly populations at release sites. However, resources did not allow all cooperators to conduct the intensive monitoring surveys needed to determine success at this level. Of the 56 releases conducted in 2002-2005, flies were found after a winter at 27 of these sites, a 48% success rate; 19 *tricuspis* sites (AL, AR, FL, GA, LA, MS, NC, PR, SC, TX) and 8 *curvatus* sites (FL, LA, NC, OK, SC, TX). In 2007 we also realized that we could no longer determine the true source of flies present in an area due to the large number of established and spreading fly populations and so the attempt to determine individual site establishment of flies was abandoned. Since 2007 the use of a phorid fly trap and a new monitoring protocol for surveying for fly presence at the county level has provided a wealth of information regarding establishment and spread of the flies. In 2009 a survey was conducted to determine the known U.S.-wide distribution of *P. tricuspis* and *P. curvatus*. Through APHIS releases, along with other federal and university groups which are also releasing flies, it was determined that *P. tricuspis* is well established in the southern areas of the IFA regulated area (AL, FL, GA, LA, MS, TX and PR), and moderately established in AR, NC and SC. To date, *P. tricuspis* is not known to be established in CA, OK or TN. The second species, *P. curvatus*, is moderately to well established in all southern IFA regulated states and PR (AL, AR, FL, GA, LA, MS, NC, OK, SC, TN, TX, and PR). *P. curvatus* has not been released in CA. Over-winter establishment of *P. obtusus* has not yet been confirmed. A publication on distribution of *P. tricuspis* and *P. curvatus* is currently being reviewed for publication and distribution maps can be found in the 2009 CPHST Annual Report.

Phorid flies will not be a stand-alone biological control agent for IFA. A homeowner will not be able to release a few flies in their back yard and see a significant decrease in IFA mounds in the yard. However, it is hoped that the flies will be an important tool in IFA management programs. It is anticipated that if several species of flies are established in an IFA infested area, the added stress caused by these flies on IFA colonies will allow native ants to compete better for food and territory. This fly-native ant-IFA interaction will hopefully allow homeowners, municipalities, and others, to make fewer chemical control product applications annually to suppress the IFA to acceptable tolerance levels, lessening the impact of the IFA on humans, livestock, wildlife and the environment.

**Table 1.** Rearing and release data for APHIS phorid fly rearing project – all species combined (*P. tricuspis, P. curvatus, P. obtusus*).

| Species | Year | No. flies produced | Approx. no. shipped* | No. field releases** | Mean flies/ release |
|---|---|---|---|---|---|
| tri,cur | 2002† | 950,063 | 58,750 | 12 | 4,895.83 |
| tri,cur | 2003 | 1,746,383 | 81,450 | 15 | 5,430.00 |
| tri,cur | 2004 | 2,280,039 | 128,602 | 12 | 10,716.83 |
| tri,cur | 2005 | 2,765,291 | 179,813 | 17 | 10,577.24 |
| tri,cur,obt | 2006†† | 2,448,798 | 178,259 | 17 | 10,485.82 |
| tri,cur,obt | 2007†† | 2,614,655 | 137,381 | 12 | 11,448.42 |
| tri,cur,obt | 2008 | 2,524,047 | 80,813 | 8 | 10,101.63 |
| tri,cur,obt | 2009 | 3,335,019 | 88,109 | 12 | 7,342.42 |
| | | | | | |
| | | | | | |
| Total | | 18,664,295 | 933,177 | 105 | |
| | | | | | |

* approx. no. potential flies shipped for release

** does not include multiple shipments to LA for initiating their own rearing facility and NM for research purposes, nor multiple shipments to cooperators for educational purposes or small research projects as flies were available

† only tricuspis shipped in 2002

†† only tricuspis and curvatus shipped in 2006 and 2007

## *Crypticerya genistae* (Soybean Scale), An Invasive Pest in Puerto Rico

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The CPHST Mission Laboratory is partnering with the PPQ Puerto Rico Work Unit and the Puerto Rico Department of Agriculture in developing a biological control project against a new invasive scale insect, *Crypticerya genistae* (Hemiptera: Monophlebidae). The insect was originally described as *Icerya genistae* by Hempel (1912) and is thought to be native to the area near Campinas, Brazil. It is very similar in appearance to cottony cushion scale (*Icerya purchasi*), but adult females have a very long extended and fluted ovisac.

The scale insect was detected in Barbados in April of 2004, attacking more than 30 different host plants (Fig. 1). The insect pest has been responsible for repeated crop losses in peanut in Barbados, and readily attacks plants in the Leguminoseae plant family, including soybeans, peanuts, and beans.

**Figure 1.** *Crypticerya genistae* on *Euphorbia* and *Leucena* sp..


**Figure 2.** Distribution of *C. genistae* in Puerto Rico.

The scale was found in two counties in southern Florida in 2005, attacking more than 50 hosts in several plant families including Convolvulaceae, Euphorbiacea, Compositae and Leguminosea. Puerto Rico discovered the presence of the scale insect in January of 2007, near a maritime port attacking wild host plants including *Mimosa* and *Euphorbia* sp. Further survey efforts during 2008 and 2009 indicate that it has spread to greater than 30 municipalities along the eastern and southern portions of the island (Figure 2), as well as the neighboring islands of Culebra and Viegues. At present, there are no current reports of significant economic losses in either cultivated crops or significant impacts to natural environments.

The scale insect is anticipated to achieve an almost island wide distribution in the near future, as a large number of suitable host plants grow wild along roadways, and several suitable host plants are found in home and commercial plantings. Two notable host plants include pigeon pea (*Cajanus cajan*) and ornamental peanut (*Arachis glabrata*), both of which are frequently found in door yard plantings in PR, and seem to be preferred host plants for the insect.

A small adventive ladybird beetle (*Anovia circumclusa*, Coccinellidae) has been identified in both Barbados and Florida as contributing significantly to the control of the pest. (Figure 3). The ladybird beetle has become fortuitously established in both Florida and Barbados along with the insect pest, but at this point has not become established in Puerto Rico. The ladybird beetle was not previously known from Barbados and it most likely hitch-hiked as eggs, larvae or adults on infested plant material (I. Gibbs, Barbados Ministry of Agriculture, Pers. Comm). Survey activities in Barbados indicates that *Rodolia cardinalis* (Coccinellidae) also attacks *Crypticerya genistae*, but in fewer numbers than *Anovia circumclusa*.

This on-going collaborative project entails:

1). Importation of *A. circumclusa* into a PR quarantine facility for screening. The Coccinellids will be screened through quarantine for 1-2 generations to make certain the insects are free of disease and hyper-parasitoids. This activity will be accomplished by Dr. Alejandro Segarra (Univ. of PR, Mayaguez).

2). Mass rearing in PR will be accomplished by the Puerto Rico Dept of Agriculture (PRDA), Pink Hibiscus Mealybug Rearing Facility.

3). Field release with evaluations for establishment and efficacy in areas affected by the pest. These activities will be accomplished by PRDA, PPQ-PR and PPQ-CPHST.

4). Follow up exploration for additional natural enemies if required in Brazil. Dr. Luiz Nogueira De Sa with EMBRAPA has agreed to collaborate in exploration of natural enemies in Campinas, Brazil. Natural enemies collected will be cultured at the Costa Lima Quarantine Facility, Sao Paulo, Brazil.

**Figure 3.** *Anovia circumclusa* (left) dorsal; (right) ventral. Photos: Divina Amalin (UFL)

## Biological Control of *Harrisia* Cactus Mealybug

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The *Harrisia* cactus mealybug, *Hypogeococcus pungens* (Hemiptera: Pseudococcidae) is a recent invasive and destructive pest of columnar cacti in Puerto Rico. Surveys since 2005 have found that *H. pungens* is rapidly spreading in Puerto Rican dry forests and is now distributed in an area of about 1,500 $km^2$ on the main island (Fig. 1). Surveys of the affected areas indicate moderate to severe infestations of several key columnar cacti (e.g., *Leptocereus quadricostatus, Pilosocereus royenii, Melocactus intortus, Stenocereus fimbratus* and *Cereus hexagonus*). The affected areas are remote and/or part of the Guanica Dry Forest Preserve, therefore chemical control options are not practical. In this case, classical biological control appears to offer the best management alternative for this pest, hopefully precluding its further spread into the continental U.S.

The *Harrisia* cactus mealybug (HCM) (*Hypogeococcus pungens* Granara de Willink) is a severe pest of columnar cacti worldwide. It is native to Northern Argentina and Chile, westernmost Brazil, Paraguay and southern Perú. In native lands, *H. pungens* also affects *Portulaca* spp. (Portulacaceae), *Acalypha* (Euphorbiaceae) and *Althernanthera* spp. (Poligonaceae). This host plant preference indicates a tendency towards polyphagy. Little is known about its biology or natural controls. In other parts of the world, *H. pungens* has been used as an effective biological control agent of cacti. In Australia and South Africa, where cacti have become rangeland nonnative invaders, the mealybug has been credited with clearing thousands of hectares of these plants. Current distribution of this species in the Caribbean includes Florida, Puerto Rico and Barbados.

Cacti are important members of the dry forests in Puerto Rico. Surveys indicate that there are 13 native and three endemic species; two columnar species are listed by the U.S. Fish & Wildlife Service as endangered: *Harrisia portoricensis* and *Leptocereus grantianus.* The cacti are important food sources for endemic bats, birds, moths and other pollinators. *Harrisia* cactus mealybug represents not only a problem for the cacti and native animals that depend on them, but there are also potential negative impacts to agriculture, precluding the establishment of commercial production of ‘dragon fruit’, *Hylocereus undatus*, an important crop for dry areas in the Caribbean. The mealybug is also a pest of ornamentals, such as *Acalypha* and *Portulaca*. The eventual expansion of *H. pungens* distribution will soon include offshore islands around Puerto Rico (Mona, Desecheo, Vieques and Culebra), which now harbor the last remaining wild populations of endangered cacti. The introduction of *H. pungens* to Puerto Rico poses a heightened extinction threat to these endemic cacti, and to the endemic organisms that depend on them.

Survey efforts in Barbados, which also has *Harrisia* cactus mealybug, indicates that HCM is readily controlled on columnar cacti by endemic parasitoids and predators. HCM is only occasionally found on ornamental plantings of *Alternanthera* and *Portulacca*, and the pest is quickly controlled by natural enemies within a few months of the initial infesta-


**Figure 1.** *Pilosocereus royenii* and areas affected by *Harrisia* cactus mealybug in Puerto Rico

**Table 1.** Natural enemies identified from HCM populations in Barbados.

| Insect Family | Natural Enemy Type | Identification |
|---|---|---|
| Cecidomyiidae | Predator | *Diadiplosis coccidivora* |
| Coccinellidae | Predator | *Diomus austrinus* Gordon |
| | | *Hyperaspis scutifera* Mulsant |
| Encyrtidae | Parasitoid | *Leptomastidea* sp nr *antillicola* Dozier |

tion. Table 1 lists the native natural enemies identified in Barbados that could potentially be used to control HCM in Puerto Rico.

In addition to the species identified in Table 1, two unknown species of parasitoids have been collected from HCM in Barbados. However, at this point in time we suspect that they may be hyper-parasitoids based on emergence characteristics from the host remains. Samples of those specimens are being forwarded to the Museum of Natural History, London, for identification.

Quarantine Host Range Testing:

Two of the natural enemies listed in Table 1 have been selected for host range screening in the University of Puerto Rico Quarantine Facility, Mayaquez, PR. Dr. Alejandro Segarra (Univ. PR) has received funds (cost share between PPQ-ER and PPQ-CPHST) to conduct host range screening for two candidate BC agents. The first candidate BC agent is *Diadiplosis coccidivora* (Cecidomyiidae: Diptera), a small midge predator of Coccidae. Our understanding of this insect's biology is that the larval forms are voracious mobile predators that feed externally on immature (egg and nymph) stages of HCM, possibly other mealybugs, and scale insects. Host range testing is necessary to determine life history and potential environmental impact parameters required to submit a "*Petition to Request the First Release of Non-Indigenous Entomophagous Biological Control Agents*" to NAPPO following "Guidelines for Petition for First Release of Non-indigenous Entomophagous Biological Control Agents (satisfying requirements of RSPM No. 12).

The second natural enemy selected for host range screening is *Leptomastidea* sp nr *antillicola* Dozier (Hymenoptera: Encyrtidae). This parasitoid appears to be a new species that has not been previously described. The pre-release evaluation of both of the natural enemies from Barbados should be achieved within one year. This includes screening against 3 to 5 non-target host mealybug species (i.e., *Maconellicoccus hirsutus, Paracoccus marginatus, Planococcus citri, and Planococcus minor*), focusing primarily on those mealybug species that have biological control management strategies already in place in Puerto Rico.

## Biological Control of Asian Citrus Psyllid

LOCATION: Mission Lab
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### Mass Production of Parasitoids

The Asian citrus psyllid (ACP), *Diaphorina citri* (Kuwayama), is the primary vector of the bacterium 'Candidatus Liberibacter asiaticus', the causal agent of Huanglongbing disease in citrus. Huanglongbing, more commonly known as greening disease, is one of the most important diseases of citrus world-wide. A key component to a management program for citrus greening disease is aggressive control of the vector ACP. *Tamarixia radiata* is recognized as the most important natural enemy of ACP in several geographic areas around the world. Currently, the CPHST Mission Laboratory is exploring rearing methods for *T. radiata* under greenhouse conditions using large orange jasmine plants. This production supports on-going research efforts to determine the effectiveness of the parasitoid in controlling ACP populations and as a seed colony for larger mass-rearing efforts. At the same time, mass-production techniques are being developed and tested at the laboratory using mature citrus trees (Fig. 1) enclosed in 12' x 12' x 10' field insectary cages. Our plans are to evaluate inundative releases of the parasitoid in the Rio Grande Valley and other ACP affected areas and to be able to transfer these technologies to state cooperators in support of area-wide ACP/greening disease management programs.

**Figure 1.** Field insectary cages being tested at the USDA citrus grove in Mission, TX, for mass-production of the biological control agent, *Tamarixia radiata*. Citrus trees are being sampled for insect population densities by technicians, Steven Rodriguez (kneeling) and Andrew Parker (standing) (Photo by Daniel Flores).

Recently, methods to produce large numbers of *T. radiata* were tested by releasing the biological control agents onto mature citrus trees infested with ACP inside field insectary cages. Tree hedging was conducted to induce the growth of new flush. Adult psyllids were attracted to the tender growth and began egg-laying. A field insectary cage was installed around each of the hedged trees and 16 female and 4 male parasitoids were then immediately released into the cage. Two months later, samples were collected and scanned under the microscope to observe parasitism rates and determine total parasitoid production. Parasitism rates in these initial low-volume trials were recorded at 19.8% and approximately 2000 adult parasitoids were produced. Through further testing, this method may prove to be an excellent tool for releasing and establishing parasitoids directly in commercial and organic groves to help suppress ACP populations. Methods to increase mass-production to 50,000 agents per tree will continue development. Availability of mass-production protocols at time of permit approval for quarantined strains of *T. radiata* will expedite the way for release, establishment, and evaluation of the new agents in citrus producing areas affected by ACP.

### Importation of Parasitoids from Pakistan

The Mission lab strain of *T. radiata* was derived from a lab strain in Florida that was initiated from collections made in Taiwan and Vietnam and subsequently evaluated and permitted for release in Florida in 1999. Parasitism rates of ACP by *T. radiata* in Florida have averaged less than 20% during spring and summer, increasing to 39-56% in the fall. Unfortunately, its effectiveness in suppressing psyllid populations under field conditions has been variable in Florida and other adjoining regions. *T. radiata* also appears to have spread to Texas along with ACP. In Texas, we have also observed parasitism rates averaging less than 20%. More recently, efforts have been made to collect and evaluate *T. radiata* from Pakistan, which has a climate more similar to that of the southern USA and where percent parasitism is reportedly much higher. In addition, diagnostic tools have been developed to discern between haplotypes of the species from differing origins in order to monitor population size during a management program where large numbers of parasitoids are released and would eventually mix with existing populations.

Arrangements were made with Abdul Rehman of CABI-South Asia to make collections of new strains of *T. radiata*. We provided packaging materials, shipping permits and labels to begin shipments to our PPQ Arthropod Quarantine Laboratory in Mission, TX. Coordination was also made with the PPQ's Plant Inspection Station in Los Indios, TX, to receive and inspect packages upon arrival into the USA and to notify us immediately upon the arrival of insects. Collections of *T. radiata* were successfully received and established in quarantine (Fig. 2) from Punjab, Pakistan in August 2009.

**Figure 2.** An adult female *Tamarixia radiata* received from Pakistan parasitizing an Asian citrus psyllid nymph. The small black wasp (0.92 to 1.04 mm long) will parasitize all 5 nymphal stages of the psyllid but has a significant preference for 5th-instar nymphs (Photo by Daniel Flores).

The small parasitic wasps will be undergoing host-specificity testing at the Mission Laboratory alongside the work being done by Dr. Mark Hoddle at University of California-Riverside. Release will be dependent on host specificity testing in quarantine to evaluate risk posed on native psyllids and non-target species as required by the permitting process. If the testing is not completed and reported, and reviewed by APHIS and NAPPO, APHIS will prohibit deliberate releases in the USA.

Establishing the Punjab strain of *T. radiata* will allow a 2nd strain for testing and release to support the ACP Biological Control Project. In addition for use in the PPQ program, *T. radiata* will be available to other researchers to support the required host specificity testing prior to release in other states where ACP is established. We are optimistic that these parasitoids will provide yet another control tool in the fight against the spread of the citrus greening disease, which is threatening the entire citrus industry.

### Future Work

Our main goals for the coming year will be to continue our host-specificity testing of *T. radiata* and to refine mass-production methods and protocols by making additional parasitoid releases on 12 to 16 mature citrus trees in field insectary cages. Further research is being planned to receive new collections of *T. radiata* from China in 2010. Studies will also be developed to determine how host-feeding impacts production, how to increase parasitism levels, how to easily harvest emerging parasitoids, and how to enhance and monitor production using day-degree models.

# Fortuitous Establishment of *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) and *Aphytis lingnanesis* (Hymenoptera: Encyrtidae) in South Texas on the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui* (Hemiptera: Diaspididae)

LOCATION: Mission Lab
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The cycad aulacaspis scale (CAS) Takagi is currently found in China, Singapore, Hong Kong, Cayman Islands, Puerto Rico, U.S. Virgin Islands, Hawaiian Islands, and Florida. It was originally described from specimens collected on a *Cycas* sp., in Bangkok, Thailand, in 1972. In recent years, finds have also been reported in California, Georgia, and Nevada. In 2006, severe outbreaks of CAS were reported in South Texas where sago palms adorn landscapes and are important ornamental plants for commercial nursery growers (Fig. 1). Of the more than 20 species of scale insects that occur on cycads in Florida, the most damaging species is CAS.

In Thailand, this armored scale is considered a pest of cycads and is usually maintained in low densities by parasitoids. In 1998, two natural enemies of CAS, a predaceous beetle, *Cybocephalus nipponicus* Endroudy-Younga (misidentified as *Cybocephalus binotatus* Grouvelle), and a parasitic wasp, *Coccobius fulvus* (Compere and Annecke), were released in Florida by the Tropical Research and Education Center - Homestead, FL. These natural enemies appear to control CAS very effectively, but the scale insect undergoes outbreaks that are more severe when the natural enemies are absent.

The predator *Rhyzobius lophanthae* Blaisdell (Coleoptera: Coccinellidae) is a natural enemy of many diaspidid scale species. Because of its prey specificity, high fecundity, adult longevity, absence of diapause, good mobility, rapid development (5-7 generations per year) and lack of parasitism, *R. lophanthae* is considered a highly important natural enemy of many armored scale species. It is known for its effectiveness in integrated pest management of scale insects via inundative releases and classical biological control programs in several countries including USA, Italy, Argentina, Bermuda, Algeria, Tunisia, Morocco and Georgia. It is also reported from Greece, where it may have spread from neighboring countries. There are no current records of this predator species ever being released in Texas.


**Figure 1.** Sago palm infested with cycad aulacaspis scale.

However, since October 2006, *R. lophanthae* has been found associated with infestations of CAS in South Texas. On July 9, 2008 insects collected in South Texas were positively identified by Dr. Natalia J. Vandenberg (USDA / APHIS / PPQ / Systematic Entomology Laboratory, Beltsville, MD) as *Rhizobius lophanthae*. All developmental stages have been observed on sago palms with larvae and adults being the most prevalent. During visual inspections, adults were found feeding on CAS or *in copulo* on cycad foliage heavily infested by CAS. Adults were found on all parts of the plants; larvae were mostly found along the midrib (top and bottom) of the fronds. Few larvae were observed on the stem of the plants. Eggs were found mostly at the base of the fronds where it meets the stem; a few times eggs were observed on the underside of the fronds close to the midrib. Pupae were found along the bases of the fronds.

While monitoring winged-male populations of CAS in South Texas, high levels of a parasitoid were observed on the yellow sticky traps at all locations. Infested fronds were collected and were placed in plexi-glass (16” x 18” x 24”) emergence cages. We found CAS was being parasitized by a wasp identified by Dr. Gregory A. Evans (USDA / APHIS / PPQ / Systematic Entomology Laboratory, Beltsville, MD) as belonging to the *Aphytis lingnanensis* group. Interestingly, *A. lingnanensis* is reported attacking the California red scale, *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) in Texas. This is the first report of an *Aphytis* species attacking *A. yasumatsui*.

Several *Aphytis* species have been used successfully in biological control programs against armored scales. For several years many scientists have searched for natural enemies of CAS but to our knowledge an *Aphytis* species was not previously found. Species in the *A. lingnanensis* group are taxonomically very similar, and difficult to separate (G.A. Evans, personal communication). The insects in South Texas may be *A. lingnanensis* Compere, which attacks several different scale species and is widespread. DNA analysis may perhaps offer the best solution to species determination.

Field data suggest that CAS populations are variable throughout the year and the establishment and abundance of both *A. lingnanensis* and *R. lophanthae* on CAS are contributing to this variability (Flores, unpublished data). No releases of these beneficial insects have been made in South Texas and no one knows exactly how they have managed to colonize this area. However, it is our belief that they fortuitously entered South Texas along with CAS on sago palms and proceeded to spread.

Since the initial discovery in the Miami area, CAS has become widely distributed in Florida. In 2006, CAS was detected in South Texas making it a new state record and suggesting that it was an incipient infestation. Our data also indicate that *Aphytis lingnanesis* and *Rhyzobious lophanthae* have become fortuitously established on CAS. Although CAS population levels vary throughout the year, to date the scale has not reached problematic population levels in South Texas.

# Biological Control of the Mediterranean Fruit Fly: Parasitoid Rearing at San Miguel Petapa, Guatemala

LOCATION: Guatemala Lab
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## Mass-production

*Fopius ceratitivorus* Wharton is a braconid parasitoid of the Mediterranean fruit fly (= medfly), *Ceratitis capitata* (Wied.). Unlike other parasitoids previously used in medfly biological control, *F. ceratitivorus* was originally collected from medfly in its purported region of origin, east Africa. Shipments of tephritid pupae from Kenya were sent to the USDA APHIS/ MOSCAMED quarantine facility at San Miguel Petapa, Guatemala, Central America in 1998. Since then rearing protocols have been developed and several phases of field evaluation have been conducted. A permit for field release of the parasitoid in Guatemala was granted in 2002. Parallel studies on the concept of combined releases of parasitoids with sterile insects in large field cages, yielded positive results. Therefore, small open field release evaluations were initiated during 2005. Additional field studies were conducted during 2006/2007. In 2008 the MOSCAMED Program began covering the cost of mass production of the parasitoid for programmatic releases. Currently, large scale releases of parasitoids combined with sterile medfly releases have been conducted as a demonstration of combined control efficacy.

The production goal in 2009 at the San Miguel Petapa facility was 1 million parasitoids/week. To accommodate the expanded production, renovations to the facility were undertaken in 2008/2009 and colonies were initially scaled back to minimum levels. In July 2009, new areas in the remodeled building were made available to locate the colony. Despite the disruptions in production, an average of 121,000 parasitoids/week was made available for field releases during 5 months of the year.

## Field releases

During 2009, field releases of *Fopius ceratitivorus* were initiated in several coffee production areas in southwest Guatemala. The areas were selected based on the interest of local action program managers to eliminate recurrent medfly detections (Fig. 1).

The test design for assessing the impact of the field releases included 3 km$^2$ plots, one km$^2$ for each of the following three treatments: (1) sterile insect releases alone; (2) sterile insect releases + parasitoids; and (3) a control without releases.

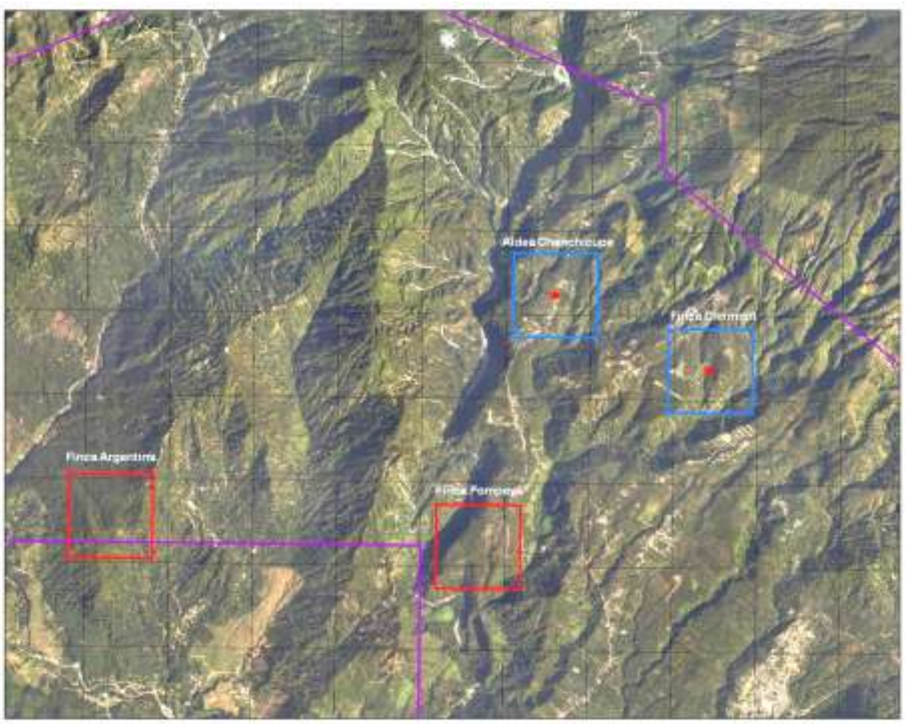

**Figure 1.** Release areas of 1 km$^2$ are delimited by red squares. Areas were subjected to combined releases of sterile males (TSL strain) and *Fopius ceratitivorus*.

Despite minimal and inconsistent production levels early in the year because of facility renovations, ground releases of parasitoids were conducted beginning in week 7. The aim of the releases was to determine infestation rates and percent parasitism from collections of coffee berries from the different plots (Fig. 2).

To be able to conduct ground field releases, a prototype insulated metal backpack (Fig. 3) was designed and is also under evaluation for maintaining the parasitoids in good condition prior to their release. The circumstantial evidence obtained was that no recurrent infestation has been present in the area were releases were conducted, although no parasitoids were recovered from the few larvae collected during the length of the test. The program however has not needed to invest additional resources in the control of those areas, despite the fact that they were considered reservoirs of the pest in previous years.

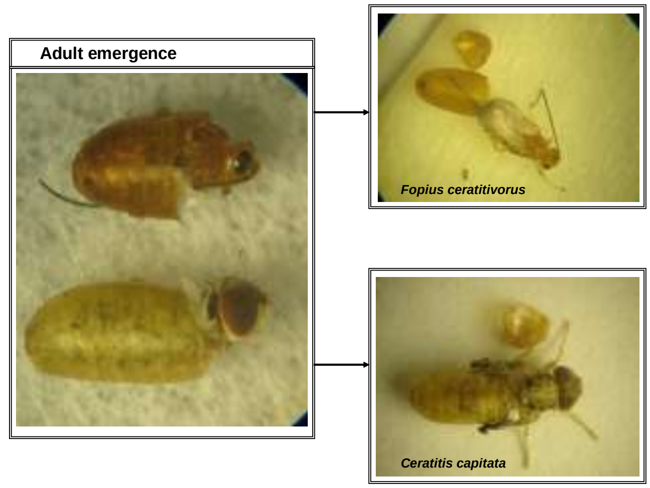


**Figure 2.** Parasitized and non-parasitized medfly pupae.

**Figure 3.** Prototype insulated backpack for the ground release of parasitoids.

## Biological Control of the Olive Fruit Fly, *Bactrocera oleae*

LOCATION: Guatemala Lab
CPHST STAFF: Pedro Rendon
CHAMPIONS: Vicky Yokoyama, ARS-CA; Charlie Pickett and Mike Pitcairn, CDFA
CONTACT: Pedro Rendon (pedro.rendon@aphis.usda.gov, 011-502-4015-1229)

### *Psytallia humilis* (Hymenoptera: Braconidae)

This parasitoid was initially collected from wild olives in Kenya in 1998 as part of a mission to find and assess new medfly parasitoids. Shipments were sent to the USDA APHIS/MOSCAMED quarantine facility at San Miguel Petapa, Guatemala and a colony has been maintain in quarantine since then. Rearing currently supports research and release efforts by ARS in California to establish this parasitoid in CA.

Under an agreement with Dr. Victoria Yokoyama, ARS Parlier, CA, the larval/pupal parasitoid *Psytallia humilis* (originally collected from Kenya) was reared in Guatemala and subsequently sent to Fresno, CA, for release and evaluation as a biocontrol agent. Because of difficulties in rearing olive fruit fly, the parasitoid is reared on irradiated medfly larvae as a factitious host. Irradiating the larvae, avoids the concern about accidental shipment of medflies to CA. Nevertheless, parasitoids are allowed to emerge, mature and after that (ca. 2 days) are placed into paraffin coated paper cups for their shipment to California.

A maintenance *P. humilis* colony of about 14,000 insects (1.3:1 male: female sex ratio) per week allowed an average of 5,700 female parasitoids/week to be shipped to California for field releases in the summer and a similar number for fall releases. These female parasitoids were shipped along with an average of 3,700 male parasitoids/week.

### *Psytallia nr. concolor*

Cooperators with CDFA, ARS and UC Berkeley found this parasitoid attacking olive fruit fly infesting wild olives in the Karsfeld region of north central Namibia. A colony was established at the Israel Cohen Biological Control Institute. Shipments were made from Israel to the Guatemala quarantine facility. *P. concolor* was sent from Israel to San Miguel Petapa, Guatemala in August 2009 (1,300 adults emerged from an original shipment of 5,000 pupae). The objective is similar that of the ARS project assessing the potential of this species as a biocontrol agent for the olive fruit fly. Currently the strain has been increased in numbers to have enough parasitoids to ship to the release sites as well as for maintenance of a good size colony to maintain a consistent shipment of the required numbers.

## The Guatemala Lab Quarantine Facility

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The Guatemala quarantine facility currently consists of ca. 931 sq. ft, although an expansion of the facility is planned for 2010. The facility has a secure padlocked entrance that leads to two dark vestibules with heavy doors. The door system allows entering the secure quarantine area only after personnel have first registered in a visitor/worker log. After registration, the first set of doors can be opened and consecutively a second set of doors that lead to the main room. Only one of the entry vestibule doors can be open at one time. The vestibules are kept dark and are maintained with clean lab coats, black light traps, air curtains and positive air pressure back into the main quarantine room. The main quarantine room also has an internal reception room, where imported material is maintained for additional biosecurity. The quarantine room has a small room for equipment/office and storage. The facility is equipped with an autoclave, biosecure sinks, an incubator, as well as general rearing supplies. The quarantine facility currently has several parasitoid strains that are being reared for release or maintained for future use. The existing colonies are as follows:

*Psytallia humilis* (Hymenoptera: Braconidae) – larval/pupal parasitoid of olive fly originally collected from Kenya.

*Psytallia nr. concolor* (Hymenoptera: Braconidae) – larval/pupal parasitoid of olive fly originally collected from Namibia.

*Psytallia cosyrae* (Hymenoptera: Braconidae) – synovigenic, koinobiont larval-pupal parasitoid of *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), and other tephritid fruit flies, including *C. capitata*. It was originally shipped to Guatemala by ICIPE in June 2002 from Kenya as 200 parasitized fruit fly pupae.

*Psytallia lounsburyii* Kenya strain (Hymenoptera: Braconidae) – larval parasitoid of olive fly. This parasitoid arrived originally in September 2009 sent from Montpelier, France. Three generations were produced, but the colony was lost during the fourth generation. A new shipment has been planned for 2010.

## The Otis Lab Quarantine Facility

CONTACT: Vic Mastro (vic.mastro@aphis.usda.gov, 508-563-9360 ext. 212)

The 2,500 square foot facility houses three laboratory rooms, three walk-in environmental chambers, a room with reach-in environmental chambers, an area for dressing and dish washing, and one room each for logs, package handling, and gas chromatography. Two autoclaves are used for waste sterilization. Movement in and out of the facility is channeled through a vestibule.

Following are the pest species in quarantine and primary focus of projects associated with these organisms in 2009:

Asian longhorned beetle (*Anoplophora glabripennis (Motschulsky])* – Insecticide testing, attractant and trap development, host testing, and development of regulatory treatments.

Emerald ash borer (*Agrilus planipennis [Fairmaire])* – Insecticide testing, including biopesticides, parasite evaluation (multiple *Spathius spp.*), production, and trap and lure development.

Sirex woodwasp (*Sirex noctilio F.*) – Development of a phenology model, production of and evaluation of *Beddingia siricidicola* (Bedding) and development of attractants and traps.

Light brown apple moth (*Epiphyas postvittana [Walker]*) – Development of regulatory treatments, and development of management treatments.

Asian gypsy moth (*Lymantria dispar [L.] and related species*) – Characterization of flight ability and inheritance of diagnostic molecular markers.

Rosy moth (*Lymantria mathura* [Moore]), Casurina Moth (*Lymantria xylina* [Swinehoe]) – characterization of flight ability and response to light.

Winter moth (*Operophtera brumata [L.]*) – Production and evaluation of parasites *Cyzenis albicans* (Fallén) and *Agrypon flaveolatum* (Gravenhorst).

## The Mission Lab Quarantine Facility

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The facility consists of a receiving lab, an identification lab, three laboratories, twelve walk-in environmental growth chambers, three reach-in environmental growth chambers, a headhouse/potting area adjacent to five separately controlled greenhouses, a pot and clothing washing area, storage area and lunchroom. One pass-through autoclave is used for waste sterilization. Movement of personnel in and out of the facility is channeled via pre– and post-entry vestibules through separate men's and women's restrooms/dressing rooms. Entry of biological materials into quarantine and exit of permitted materials out of quarantine is conducted through a secured-access pass-through chamber.

In 2009, the facility supported the following APHIS and ARS programs:

BIOLOGICAL CONTROL PROGRAMS

APHIS

Asian citrus psyllid (*Diaphorina citri* (Kuwayama) (Homoptera: Psyllidae)) - Receipt of 4 shipments of the parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), from Punjab, Pakistan, collected by CABI South Asia. Quarantine processing and establishment of cultures for host specificity testing and efficacy evaluation.

Russian knapweed (*Acroptilon repens*) - Receipt of shipments of the parasitoid, *Jaapiella ivannikovi* (Diptera: Cecidomyiidae) from CABI, Delamont, Switzerland, for quarantine processing and establishment of cultures to support field releases by the CPHST Fort Collins lab.

ARS

Giant reed (*Arundo donax)* - Maintenance of cultures of 4 species of natural enemies for host specificity testing.

OTHER APHIS ACTIVITIES:

In addition, the facility housed activities for other projects related to:

Mexican fruit fly (*Anastrepha ludens*) - Maintenance and evaluation of wild cultures of Mexican fruit fly (MFF) for eventual introduction into the MFF mass rearing facility for strain replacement.

Asian citrus psyllid:

- Dense Interplantings of Grapefruit and Guava
- Area-wide Management of Asian Citrus Psyllid -

# PUBLICATIONS

Peer-Reviewed


Barr, N.B., D.G. Hall, A.A. Weathersbee III, R. Nguyen, P. Stansly, J.A. Qureshi, and D. Flores. 2009. Comparison of laboratory colonies and field populations of *Tamarixia radiata*, an ecto-parasitoid of the Asian Citrus Psyllid, using ITS and COI DNA sequences. Journal of Economic Entomology 102(6): 2325-2332.

Bloem, K., P. Greany, and J. Hendrichs, J. (Eds.). 2009. Use of Radiation in Biological Control, 362 pp. Biocontrol Science and Technology 19: Supplement 1 (Special Issue).

Cancino, J., L. Ruíz, J. Hendrichs, and K. Bloem. 2009. Evaluation of sequential exposure of irradiated hosts to maximize the mass rearing of fruit fly parasitoids. Biocontrol Science and Technology 19: 95-109.

Flores, D., and J. Carlson. 2009. Fortuitous Establishment of *Rhyzobius lophanthae* (Coleoptera: Coccinellidae) and *Aphytis lingnanesis* (Hymenoptera: Encyrtidae) in South Texas on the Cycad Aulacaspis Scale, *Aulacaspis yasumatsui* (Hemiptera: Diaspididae). Southwestern Entomologist Scientific Note 34(4): 489-492.

Flores, D., D.G. Hall, D.A. Jenkins, and M. Sétamou. 2009. Abundance of Asian citrus psyllid on yellow sticky traps in Florida, Puerto Rico and Texas. Southwestern Entomologist 34 (1): 1-11.

Gomez, N., R.C. Venette, J. Gould, and D. Winograd. 2009. A unified degree day model describes survivorship of *Copitarsia corruda* Pogue & Simmons (Lepidoptera: Noctuidae) at different constant temperatures. Bulletin of Entomological Research 99: 65-72.

Hendrichs, J., K. Bloem, G. Hoch, J. Carpenter, P. Greany, and A. Robinson. 2009. Improving the cost-effectiveness, trade and safety of biological control for agricultural insect pests using nuclear techniques. Biocontrol Science and Technology 19: 3-22.

Juang-Horng Chong, J.J. Tefel, A.L. Roda, and C.M. Mannion. 2009. Effects of Beetle Density and Time of Day on the Dispersal of Gratiana boliviana (Coleoptera: Chrysomelidae). Florida Entomologist 92(1): 99-106.

Long, S.J., D.W. Williams, and A.E. Hajek. 2009. *Sirex* species and their parasitoids in *Pinus sylvestris* in eastern North America. Canadian Entomologist 141: 153-157.

Nielsen, C., D.W. Williams, and A.E. Hajek. 2009. Putative source of the invasive *Sirex noctilio* fungal symbiont, *Amylostereum areolatum*, in the eastern United States and its association with native siricid woodwasps. Mycological Research 113: 1242-1253.

Overholt, W.A., R. Diaz, K.L. Hibbard, A.L. Roda, D. Amalin, A.J. Fox, S.D. Hight, J.C. Medal, P.A. Stansly, B. Carlisle, J.H. Walter, P.J. Hogue, L.A. Gary, L.F. Wiggins, C.L. Kirby, and S.C. Crawford. 2009. Releases, Distribution and Abundance of *Gratiana boliviana* (Coleoptera: Chrysomelidae), a Biological Control Agent of Tropical Soda Apple (*Solanum viarum*, Solanaceae) in Florida. Florida Entomologist 92 (3):450-457.

Peña, J.E., J.C.V. Rodrigues, A. Roda, D. Carrillo, and L.S. Osborne. 2009. Predator-prey dynamics and strategies for control of the red palm mite (*Raoiella indica*) (Acari: Tenuipalpidae) in areas of invasion in the Neotropics. IOBC/wprs Bulletin 50: 69-79.

Tomic-Carruthers, N. 2009 Rearing *Hylobius transversovittatus* and *Cyphocleonus achetes* larvae on artificial diets (Coleoptera: Curculionidae). Florida Entomologist 92 (4): 656-657.

Programmatic or In-house


Hansen, R.W. 2009. Current status of, and future prospects for, biological control of musk thistle (*Carduus nutans*) and Scotch thistle (*Onopordum acanthium*) in the US. USDA-APHIS-PPQ. White Paper. 42 p.

Gould, J., I. Fraser, Z. Yang, X. Wang, V. Mastro, and D. Williams. 2009. Update on EAB Biocontrol: Release and recovery of *Spathius agrili* and discovery of new potential biocontrol agents in Asia. *In* Proceedings of the USDA Interagency Research Forum on Gypsy Moth and other Invasive Species. Annapolis, MD. January 2009.

Keena, M., J. Gould, and L. Bauer. 2009. Factors that influence Emerald Ash Borer (*Agrilus planipennis*) adult longevity and oviposition under laboratory conditions. *In* Proceedings of the USDA Interagency Research Forum on Gypsy Moth and other Invasive Species. Annapolis, MD. January 2009.

Williams, D.W., and V.C. Mastro. 2009. Evaluation of *Beddingia siricidicola* as a biological control agent of *Sirex noctilio* in North America. In: Proceedings. 19th U.S. Department of Agriculture interagency research forum on invasive species. Gen. Tech. Rep. NRS-P-36. USDA Forest Service, Newtown Square, PA.

Williams, D.W., K.E. Zylstra, and V. Mastro. 2009. Biological control of *Sirex noctilio* in North America by *Beddingia siricidicola*: 2008 update. In: Proceedings. 20th U.S. Department of Agriculture interagency research forum on invasive species. Gen Tech Rep NRS-P-51 USDA Forest Service, Newtown Square, PA.

# Biological Control Related Cooperative/Interagency Agreements Managed by or Partnered with CPHST Scientists

Target: Canada Thistle
Title: Identification of eriophyid mites on native and weedy *Cirsium* thistles in North America (using molecular diagnostics)
Cooperator: John Lydon, ARS (Interagency Agreement)
Funding: $7,000 – BC Line
ADODR: Richard Hansen
Objective: Identification of eriophyid mites on native and weedy Cirsium thistles in North America to help determine the host specificity and safety of the Canada thistle rust mite, *Aceria anthocoptes*, in the US.

Target: Hawkweeds
Title: Collection and propagation of native plants for use in host specificity screening with prospective hawkweed biological control agents
Cooperator: Mark Schwarzlaender, University of Idaho, Moscow, ID
Funding: $7,500 – BC Line
ADODR: Richard Hansen
Objective: Collection and propagation of native plants for use in host specificity screening with prospective hawkweed biological control agents to ensure their efficacy and safety.

Target: Yellow Toadflax
Title: Development of rearing techniques for *Mecinus janthinus* on yellow toadflax
Cooperator: Andrew Norton, Colorado State University, Ft. Collins, CO
Funding: $8,094 – BC Line
ADODR: Richard Hansen
Objective: Assess the relative utilization of yellow toadflax vs. Dalmation toadflax by the stem mining weevil *Mecinus janthinus* and develop methods to rear the weevil on caged plants.

Target: Exotic Weeds
Title: Development of weed biological control agents
Cooperator: Hariet Hinz, CABI Europe-Switzerland, Delémont, Switzerland
Funding: $204,139 – BC Line
ADODR: Richard Hansen
Objective: Pre-release development and host specificity testing of biological control agents of:

- Canada thistle
- Dyer's woad
- Field bindweed
- Garlic mustard
- Hoary cress
- Hound's-tongue
- Perennial pepperweed
- Russian knapweed
- Yellow toadflax

Target: Cycad Scale
Title: Methods development for a predator and a fungus for biological control of the cycad aulacaspis scale
Cooperator: Ron Cave, University of Florida, Ft. Pierce, FL
Funding: $15,000 ($7,500 ER / $7,500 CPHST) – BC Line
ADODR: Ron Weeks, ER (Dan Flores, CPHST Cooperator)
Objective: Host range testing and development of rearing methods of a new un-described ladybeetle *Phaenochilus sp.* and the entomopathogenic fungus *I. fumosorosea* for potential use against the cycad scale.

Target: EAB
Title: *Spathius agrili* Yang (Braconidae) field release for control of emerald ash borer in Michigan: Non-target ef fects study
Cooperator: John S. Strazanac, West Virginia University
Funding: 56,100 – EAB Program
ADODR: Juli R. Gould
Objective: Post-release monitoring of the exotic EAB biological control agent *Spathius agrili* for non-target impacts. Three species of wood borers native to North America that were used in laboratory host range testing are the focus of this field study, including the bronze birch borer (*Agrilus anxius* Gory), two-lined chestnut borer (*Agrilus bilineatus* (Weber), and red-headed ash borer (*Neoclytus acuminatus* (F.).

Target: EAB
Title: Biocontrol of emerald ash borer: Exploration and research in China
Cooperator: Yang Zhong-qi, Chinese Academy of Forestry, Beijing, China
Funding: $13,000 – EAB Program
ADODR: Juli R. Gould
Objective: Collect and send additional specimens of *Spathius agrili* to increase genetic diversity for mass-rearing and continue investigating natural enemies that attack EAB at low population densities.

Target: EAB
Title: Ecology and natural control of *Agrilus planipennis* in South Korea
Cooperator: Professor Kuo Duok Park, Dongguk University, Seoul, South Korea
Funding: $15,030 – EAB Program
ADODR: Dave Williams
Objective: To identify, collect and study natural enemies of EAB from South Korea.

Target: *Harrisia* Cactus Mealybug
Title: Pre-release quarantine evaluations of natural enemies for the biological control of harrisia cactus mealy bug
Cooperator: Alejandro Segarra, University of Puerto Rico, Mayaguez
Funding: $44,000 ($29,000 ER / $15,000 CPHST) – BC Line
ADODR: Leyinska Wiscovitch (Matt Ciomperlik CPHST Cooperator)
Objective: Pre-release quarantine evaluations of natural enemies for biological control of the harrisia cactus mealy bug, including determining the biology, life history, host range and potentially adverse environmental im pacts of the imported natural enemies *Diadiplosis coccididvora* (Diptera: Cecidomyiidae) and *Leptomasti dae sp.* Hymenoptera: Encyrtidae).

Target: Imported Fire Ant
Title: Biocontrol of the imported fire ant
Cooperator: Jason Byrd, FL DOACS Division of Plant Industries
Funding: $287,500 ($167,500 ER / $20,000 WR / $100,000 CPHST) – BC Line (Regions) and IFA Line (CPHST)
ADODR: Paul Hornby (Ann-Marie Callcott CPHST Cooperator)
Objective: To mass-rear and release permitted phorid fly species throughout IFA-infested states.

Target: Red Palm Mite
Title: Classical biological control of the red palm mite
Cooperator: Marjorie Hoy, University of Florida, Gainesville, FL
Funding: $27,400 ($17,400 ER / $10,000 CPHST) – BC Line
ADODR: Paul Hornby (Amy Roda CPHST Cooperator)
Objective: To evaluate and conduct host range testing of a new strain/species of predatory phytoseiid mite collected from Mauritius on RPM and determine its relationship to a similar species *A. largoensis* already in Florida.

Target: Exotic Invasive Pests
Title: Development of biological control and other safeguarding tools to manage invasive pests
Cooperator: Moses Kairo, Florida A&M University, Tallahassee, FL
Funding: 200,000 – Office of the APHIS Administrator
ADODR: Ken Bloem
Objective: Six projects were supported in 2009, including:

- Improving offshore mitigation strategies for invasive pests coming from the Caribbean and Central America (Continuation and Expanded Project)
- Development of LUCID Keys (Continuation Project)
- Development of protocols for risk assessment and management for agents used in classical and augmentative biological control (Continuation Project)
- Student internships and recruitment at CESTA and the Center for Biological Control
- Economic impact of cogongrass in southeastern United States (Continuation Project) and other economic aspects of invasive species (New Activity)
- Use of geospatial technologies to understand invasion processes and to mitigate invasive species (New Activity)

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